# Dihydrotestosterone and Bicalutamide Do Not Affect Periostin Expression in Androgen-dependent LNCaP Prostate Cancer Cell Lines

FRANCESCA ARGELLATI[1], PIER VITALE NUZZO[1], FRANCESCO RICCI[1], ROSA MANGERINI[2], ALESSANDRA RUBAGOTTI[1,2] and FRANCESCO BOCCARDO[1,2]

[1]*Department of Internal Medicine, School of Medicine, University of Genoa, Genoa, Italy;*
[2]*IRCCS San Martino University Hospital – IST National Cancer Research Institute: Academic Unit of Medical Oncology (Medical Oncology B), Genoa, Italy*

**Abstract.** *Background/Aim: To investigate periostin (POSTN) expression in the LNCaP cell line. Materials and Methods: Our LNCaP strain did not constitutively express the POSTN gene. Through cell transfection with a cloning vector, we developed an LNCaP cell line that stably expressed POSTN. LNCaP wild-type and transfected cells were incubated with dihydrotestosterone (DHT) in the presence/or absence of bicalutamide (BIC). POSTN mRNA was detected by quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) and growth was measured with the MTT assay. Results: POSTN transfection stimulated LNCaP cell growth. While POSTN transfection did not interfere with the stimulatory effect of DHT, BIC had an inhibitory effect on cell proliferation. However, exposure to either DHT and/or BIC was not able to interfere with POSTN expression per se. Conclusion: We confirmed the role of POSTN in promoting cancer cell growth. Although POSTN transcription is not likely to be androgen-dependent, the fact that increased cell proliferation POSTN-mediated was impaired by BIC suggests an androgen modulation of POSTN interaction proteins.*

Prostate cancer (PCa) has become the most common malignancy among men in most Western countries (1). This tumor is under the pivotal driving of androgens, which not only play an important role in the development and function of the prostate but are also intimately involved in the development and progression of PCa (2). Even when PCa is apparently confined to the prostate, it encompasses a broad spectrum of diseases, some of which are characterized by an extremely indolent behavior and others by a very poor outcome. Therefore, an important clinical question is how to recognize patients who require aggressive treatment at this stage and to distinguish them from those who can be safely managed within observational programs (3).

Many studies have focused on identifying individual gene or protein candidates with a potential causative role in prostate carcinogenesis with the hope of identifying novel tumor markers that might represent more efficient diagnostic and prognostic tools and novel therapeutic targets (4). Many of the cellular abnormalities present in solid tumors are structural in nature and involve either the nuclear matrix (NM) or the proteins of the extracellular matrix (ECM) (5, 6).

Among the components of ECM, increasing interest has been focused on periostin (POSTN), a 93.3 kDa secreted protein that promotes integrin-dependent cell adhesion and motility. Up to six different splice isoforms of POSTN have been reported, four of which have been fully-sequenced and annotated (7, 8). The isoforms of POSTN are between 83 and 93 kDa in mass and differ in their *C*-terminal sequences, characterized by individual presence or absence of cassette exons 17 21 (UniProtKB/Swiss-Prot, March 2011) (9). Structural variations of *C*-terminal domain are capable of modulating the function of POSTN in the context of the ECM and it is possible that they influence the morphology and functional changes associated with the epithelial mesenchymal transition (EMT) (9, 10).

Recent studies appear to suggest that POSTN might play an important role in tumor development since it is up-regulated in a wide variety of cancer types, including PCa (9, 11, 12). In PCa, POSTN has been shown to be highly

*Correspondence to:* Professor Francesco Boccardo, MD, IRCCS San Martino University Hospital – IST National Cancer Research Institute and the University of Genoa, Academic Unit of Medical Oncology (UOC Oncologia Medica B), Largo Rosanna Benzi 10, 16132 Genoa, Italy. Tel: +39 0105600560, Fax: +39 010352753, e-mail: f.boccardo@unige.it

*Key Words:* Periostin, prostate cancer, LNCaP cell line, androgens, antiandrogens.

overexpressed in tumor tissues more than in healthy peritumoral tissues, especially in high-grade cancer, and to be mainly overexpressed in tumor stroma (11). However POSTN has been shown to be overexpressed both by epithelial and stromal cells in another study, in which a strong association of epithelial expression with local tumor stage was observed, while stromal expression appeared to be correlated mainly with high Gleason score phenotypes and an increased risk of biochemical failure (12).

Recent work by our group analyzed a set of tissue samples obtained from patients submitted to radical prostatectomy; in this study POSTN expression was significantly higher in tumor stroma, and was associated with shorter survival (13). However POSTN overexpression was also observed in epithelial cells, where, by contrast, it appeared to be associated with longer PSA progression-free survival (13). Moreover, patients with both high stromal and low epithelial expression made up a subgroup with a very bleak prognosis (13). These studies suggest that POSTN might play different functional roles in different phases of tumor progression on the basis of its compartmentalization.

How POSTN expression is regulated in the tumor microenvironment remains substantially unexplored and little is known about the molecular cross-talk between cancer and stromal cells and how the tumor microenvironment is involved in modulating POSTN synthesis.

In order to further clarify the role of POSTN in PCa progression, the present study was performed to investigate POSTN expression in the human androgen-dependent PCa cell line LNCaP to evaluate whether and how overexpression of this protein might influence tumor cell proliferation *in vitro*, and whether, and to what extent, this process is under androgenic control.

## Materials and Methods

*Cell culture conditions*. The human androgen-dependent LNCaP cell line was purchased from the American Type Culture Collection Promochem s.r.l (ATCC/LGC, Manassas, VA, USA) . Milan, Italy. The human fibroblast cell line MF3T, which served as control, was kindly donated by Dr R. Benelli (IRCCS San Martino University Hospital – IST National Cancer Research Institute, Genoa, Italy). Cell lines were cultured in monolayer in RPMI-1640 (PAA, Laboratories GmbH: Pasching, Austria, Europe) supplemented with 10% fetal bovine serum, 100 U/ml of penicillin, and 50 mg/ml streptomycin at 37˚C in an atmosphere of 5% $CO_2$ in an incubator. The cells were sub-cultured once a week.

*Transfection, cell growth and experiments*. Because the LNCaP cell line we used did not constitutively express *POSTN*, we developed a LNCaP cell line that stably expressed the *POSTN* gene, through cell transfection with a cloning vector (pCMV-XL5/POSTN; OriGene, Rockville, MD, USA) containing the *POSTN* gene. LNCaP cells transfected with the pCMV-XL5 empty vector (OriGene) were used as negative control. The turbofectin transfection procedure was performed according to the manufacturer's instructions. LNCaP cells were seeded at $1\times10^6$ cells in a six-well culture plate, 24 hours prior to transfection. For both plasmids, transfection was performed with 1.5 μg DNA per well.

Our experiments were performed in phenol red-free RPMI medium supplemented with 10% charcoal-treated fetal calf serum (FCS), 2 nM L-glutamine and 1% Penicillin/Streptomycin (PS). Log-phase growing cells were harvested from 75-$cm^2$ flasks by trypsinization; for *POSTN* experiments and modulation, cells were loaded into a 25-$cm^2$ flasks at a final density of $1\times10^6$ cells. After 48 h, the medium was changed and control cells were incubated with fresh phenol red-free medium only or containing 0.1 nM dihydrotestosterone (DHT) for 72 h. Subsequently, the former were incubated for a further 72 h with bicalutamide (BIC) 10 μM, the latter were incubated for a further 72 h in the presence/or absence of BIC. In each case, the total incubation time was 144 h. All experiments were run in triplicate.

*Quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR)*. To evaluate whether transfection would result in an effective gene expression, quantitative real-time reverse transcription-polymerase chain reaction was employed. Total RNA isolation from cells was achieved using Trizol reagent according to the manufacturer's instructions (Invitrogen, Paisley, UK). The reverse transcription reaction was carried out with 1 μg of RNA using Super Script™ II Reverse Transcriptase kit (Invitrogen, Paisley, UK) and oligo dT primers (Invitrogen), in a final volume of 20μl. The forward and reverse primers were synthesized and were as follows: *POSTN* forward: 5'GCAAACAGCTCAGAGT CTTCGT3', and reverse: 5'CAGCTTCAAGTAGGCTGAGGAA3'; glyceraldehyde-3-phosphate-dehydrogenase (*GAPDH*) forward: 5'GAAGGTGAAGGTCGGAGT3', and reverse: 5'CATGGGTGG AATCATATTGGAA3'. All primers for the tested genes were designed using primer3 software (http://frodo.wi.mit.edu/primer3/input.htm).

After an initial denaturation step of 10 min at 94˚C, 30 cycles of 1 min at 94˚C followed by 45 s at 56˚C and 1 min at 72˚C were performed, followed by a final extension at 72˚C for 10 min.

qRT- PCR was carried out on a Light Cycler® 480 System II (Roche, Basel, Switzerland) using Light Cycler® 480 SYBR Green I Master. After amplification, melting curves were carried out to monitor amplicon identity. Expression data were normalized to *GAPDH* gene expression data, obtained in parallel. Relative expression values with standard errors were determined using Qgene software. Expression changes were calculated using the mean value of normalizations obtained using *GAPDH* gene as reference.

*Western-blot analysis*. In order to check whether transfected POSTN gene was expressed as protein, Western-blot analysis of total proteins was performed. Total proteins were extracted from cells using the RIPA buffer (SIGMA, St. Louis, Missouri, USA) containing a protease inhibitor cocktail (SIGMA, St. Louis, Missouri, USA); the quantification of the proteins was performed using the BCA method (Bio-Rad Laboratories, Hercules, CA, USA). 30 ug of cell lysate were separated on 10% SDS-PAGE and then transferred into a PVDF membrane. The membrane was blocked with 5% non-fat dry milk containing 0.1% Tween-20 for 1h at room temperature. Anti-periostin primary antibody (Novus Biologicals, Littleton, CO, USA) was incubated for 2h at room temperature, washed with PBS/0.1% Tween-20, probed with secondary antibody

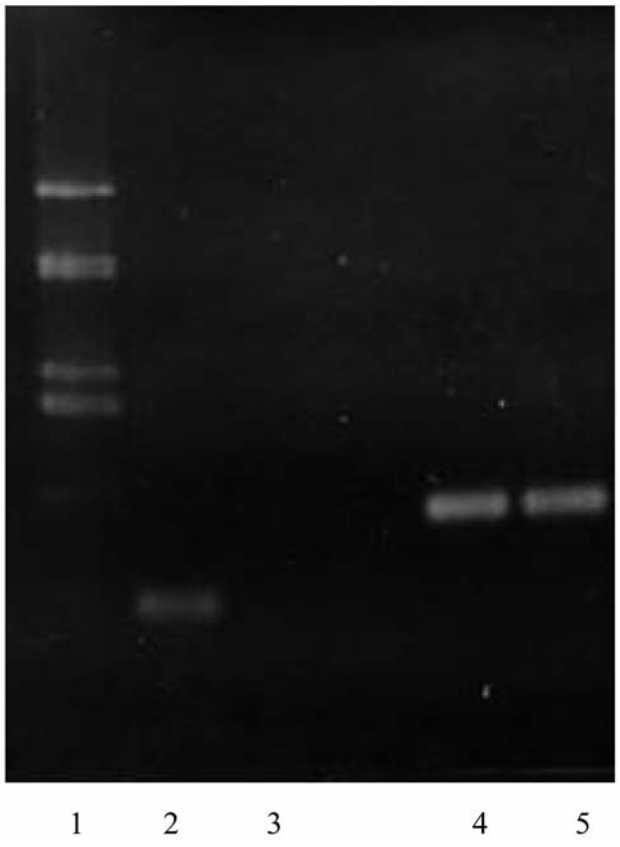


Figure 1. *Expression of periostin (POSTN) gene in LNCaP and MF3T cell lines. Quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) analysis showed that POSTN mRNA was not detected in the LNCaP cell line, but only in the MF3T cell line, used as control. Expression data were normalized to that for glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) gene expression, obtained in parallel. Relative expression values with standard errors were calculated using Qgene software. Expression changes were calculated using the mean value of normalizations obtained using GAPDH gene as references. 1 Molecular weight marker; 2 POSTN in MF3T cells; 3 POSTN in LNCaP cells; 4 GAPDH in MF3T cells; 5 GAPDH in LNCaP cells.*

coupled with peroxidase and detected by enhanced chemio-luminescence (ECLPlus, Ge-Healthcare, Niskayuna, NY, USA).

*Cell viability assay.* Cell viability was evaluated thorugh cell proliferation assessment in the different colture conditions. Cell proliferation was measured with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-ltetrazolium bromide (MTT; Roche) method. One hundred microliters of cells were seeded in a 96-well plate at a density of $4\times10^4$ cells per well and were subsequently incubated for 24 h to allow cell attachment. After incubation for 72 h with treatments, 10 μl of MTT solution [5 mg/ml in Phosphate Buffered Saline (PBS)] were added to the wells and the cells further incubated for 4 h, then the lysis solution (100 μl for well) was added and the plate was left to incubate overnight. The absorbance was measured the following day at 570 nm on an enzyme-linked immunosorbent assay (ELISA) reader.

*Statistical analysis.* Student's *t*-test was used to assess statistical significance between means. Differences between means were considered significant when $p<0.05$. All statistical analyses were carried out with the Graph Pad Prism 4 software (Graph Pad Software, Inc., La Jolla, CA, USA).

## Results

*POSTN gene and protein expression in LNCaP and MF3T cell lines.* RT-PCR analysis showed that *POSTN* mRNA was not detected in LNCaP cell line, but only in the MF3T cell line, used as control (Figure 1). Accordingly POSTN protein was expressed in the MF3T cell line but not in LNCaP wild-type cell line. However it was expressed in the LNCaP cells transfected with the gene, proving the adequacy of the model. Noteworthy the cells transfected only with the empty vectors did not express the protein (Figure 2).

*Effects of DHT and BIC on cell growth.* The LNCaP cell line we used was the FGC subline, which expresses the androgen receptor (AR), is androgen-dependent for growth but does not express the estrogen receptor (ER).

Our experiments showed that LNCaP cell proliferation was sustained by the addition of DHT ($p<0.05$ DHT *vs.* no supplementation). BIC is a non-steroidal anti-androgen commonly employed in the treatment of PCa, either in combination with LH-RH analogs or as a single therapy in selected cases (14, 15), and which has been shown to retain anti-androgenic properties in this cell model (16, 17). The exposure of cells to this anti androgen did not affect tumor growth *per se*; however, the addition of BIC did inhibit DHT-stimulated tumor growth (Figure 3), confirming the androgen-dependency of the cell-line strain available to us. Because our LNCaP strain did not constitutively express POSTN, in order to mimic the effect of *POSTN* gene expression in PCa cells *in vitro*, we transfected cells with a cloning vector expressing the *POSTN* gene, obtaining a LNCaP/POSTN cell line that stably expresses the *POSTN* gene.

Ectopic expression of *POSTN* appeared to stimulate LNCaP cell growth in a manner comparable to that induced by treatment with DHT, although in this case, differences with respect to untreated cells were not statistically significant. While transfection with the *POSTN* gene did not interfere with the stimulatory effect of treatment with DHT (Figure 3), treatment with BIC had an inhibitory effect on cell growth, both in the presence and in the absence of DHT ($p<0.0001$).

*Effects of DHT and BIC on POSTN gene expression.* In order to better-understand the effects of androgens and anti-androgens on the proliferation of cells transfected with the *POSTN* gene, we also investigated whether the treatment with DHT and BIC, either alone or in combination, could modulate *POSTN* mRNA expression. As is clear from Figure

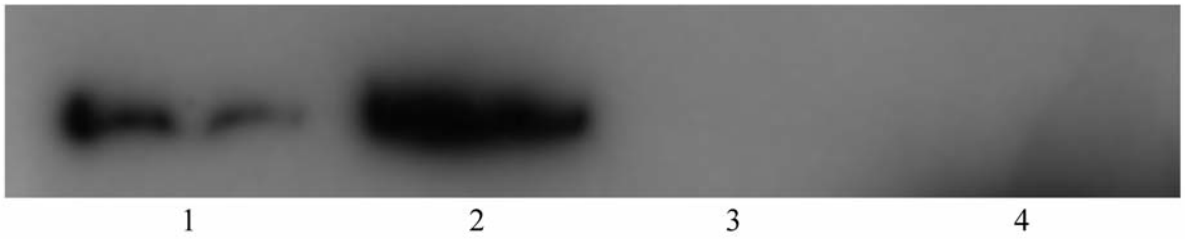


Figure 2. *Expression of periostin (POSTN) protein in LNCaP and MF3T cell lines. The western-blot analysis showed that POSTN was not detected in the LNCaP wild-type and in the LNCaP transfected with empty vector, but only in the MF3T cell line, used as control, and in the LNCaP transfected with POSTN gene. 1 POSTN in MF3T cells; 2 POSTN in LNCaP cells transfected with POSTN gene; 3 POSTN in LNCaP wild-type; 4 POSTN in LNCaP cells transfected with empty vector.*

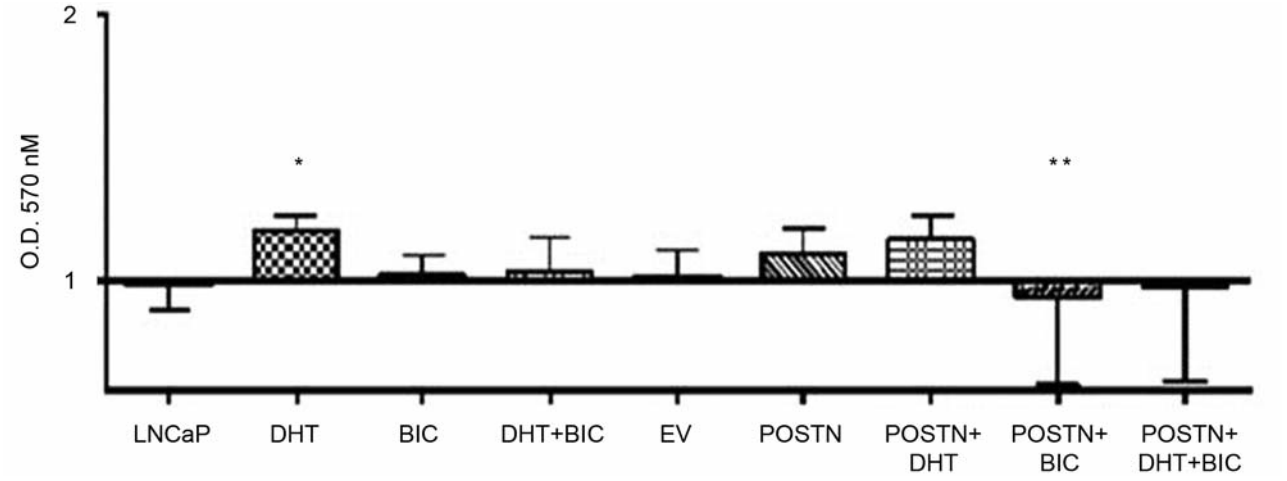


Figure 3. *Effects of dihydrotestosterone (DHT) and Bicalutamide (BIC) on cell growth. LNCaP wild-type cell line and LNCaP cell line transfected with a cloning vector (pCMV-XL5/POSTN; OriGene,Rockville,MD,USA) containing Periostin (POSTN) gene were incubated in RPMI-1640 phenol-red free medium (PAA, Linz, Austria) supplemented with 10% charcoal-treated FCS. Cell were then treated with DHT;0,1 mM and/or BIC; 10 μM. LNCaP cells transfected with the pCMV-XL5 empty vector (OriGene) were used as negative control. Cell proliferation was measured with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-ltetrazolium bromide (MTT, Roche) method. (*p<0.05, DHT compared to LNCaP, **p<0.0001 BIC+POSTN compared to DHT+POSTN).*

4, *POSTN* mRNA expression did not appear to be influenced by DHT or BIC, suggesting that these drugs do not interfere directly with transcription of the *POSTN* gene.

## Discussion

The ECM is a key component of the tumor microenvironment and significantly influences the biology of the tumor and its proliferation by providing factors for cell growth and survival, and by stimulating neo-angiogenesis (18, 19).

POSTN, a major ECM component, is thought to play a key role in cell adhesion and osteoblast recruitment (7); it has been shown to interact with other ECM proteins, such as fibronectin, tenascin-C, collagen-V, and to maintain tissue homeostasis and cell adhesion. However little is known about the POSTN interaction network. Similarly, there is still limited knowledge about the genetic and epigenetic factors which modulate up-regulation/de-regulation of POSTN and its accumulation in tumor stroma. Moreover, little is known about the up-regulation of POSTN by epithelial cells, as well as the biological function according to the different localization of this protein.

Several studies have indicated that *POSTN* mRNA and protein are not usually expressed in epithelial cancer cell lines. In the present study we also failed to document *POSTN* expression in the hormone-dependent LNCaP cell line. Our findings are apparently in contrast with those reported by Sun *et al.* (20), who analyzed POSTN expression in two hormone-refractory PCa cell lines (DU145 and PC3) and two hormone-dependent PCa cell lines (22RV1 and LNCaP), showing that LNCaP, but not 22RV1 and the two androgen-independent lines, do express *POSTN* mRNA and protein. The discrepancy

between our own findings and those of the Chinese investigators in regard to the LNCaP cell line might depend on the different strain available to our groups. The LNCaP cell line used in our study was the FGC subline, which, comparably to the 22RV1 line, is androgen-dependent for growth, expresses the AR but does not express the ER. Unfortunately the Chinese investigators do not provide details in their article about the LNCaP cell line strain used for their experiments. However, it is likely, at this point, that the two strains differ from each other in their phenotype, including their ability to express POSTN on a constitutive basis.

Because our cells were lacking constitutive *POSTN* gene, as previously described in detail, we generated a cell model expressing the *POSTN* gene, after transiently transfecting cells with the gene. It is interesting to note that gene transfection resulted in cell proliferation, thus providing experimental evidence that POSTN expression in epithelial cells might commit them to tumor progression, a process that, as was mentioned in the preliminary remarks, might be correlated with different biological mechanisms, including the activation of the Phosphoinositide 3-kinase/serine threonine-specific protein kinase (PI3-K/Akt) pathway or the more complex EMT.

The apparent discrepancy between these data and the results of our previous work, in which, by using a polyclonal antibody, we evaluated POSTN expression in the epithelium and stroma of paraffin-embedded tumor samples, showing that POSTN epithelial overexpression is correlated with a lower risk of biochemical progression, might be possibly explained in view of the postulated difference in the role played by the different POSTN isoforms (13). Preliminary findings obtained in a subset of our paraffin-embedded samples, using two recombinant monoclonal antibodies able to recognize specific POSTN isoforms, appear to suggest that different POSTN isoforms may localize differently in the epithelium and stroma, and, at the epithelial level, in the nucleus rather than in the cytoplasm. In the present work, we were not able to characterize the isoform of the protein encoded by the transfected *POSTN* gene. Thus, we cannot rule-out that epithelial expression of POSTN might have a promoting effect on proliferation, as was the case of the *POSTN* gene transfected in the LNCaP cells in the present study, or an inhibitory effect, as might be the case of the isoform prevalently localized in the nucleus rather than in the cytoplasm that we documented in our previously mentioned study on archival samples (13). Unfortunately, in the present study we were not able to investigate other functional aspects, beyond tumor proliferation, possibly controlled by *POSTN* transcription.

The exposure to either DHT or BIC did not *per se* interfere with the expression of the *POSTN* gene; this fact appears to suggest that gene transcription is not likely to be under androgenic control. The present data, however, do not exclude that the androgen receptor could nonetheless drive *POSTN* transcription through mechanisms different from the

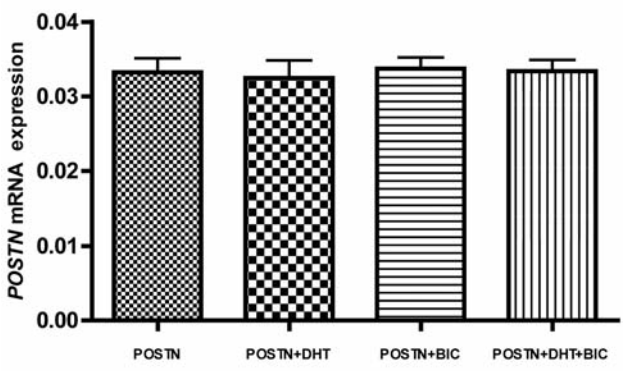


Figure 4. *Effects of dihydrotestosterone (DHT) and Bicalutamide (BIC) on periostin (POSTN) gene expression. No significant change in the level of POSTN mRNA expression dected by quantitative real-time PCR was observed after 48 hours of treatment with 0.1mM DHT and BIC 10 μM, alone and in combination.*

interaction with its ligand. The fact that cell proliferation induced by *POSTN* transfection could be almost completely reverted by the concurrent administration of BIC, rather suggests that at least one of the putative interactors of POSTN in these experimental conditions might be under androgenic control. Because we confirmed that the transfection of LNCaP cells with the *POSTN* gene can induce cell growth and that this effect can be partly reverted by the concurrent administration of BIC, it might be expected that gene silencing should also result in the inhibition of tumor growth. Indeed, this was the case in the elegant experiment performed by Sun *et al*. in an LNCaP strain constitutively expressing the *POSTN* gene (20). Through the transfection of cells with shRNA-POSTN lentiviral particles, these investigators showed that the protein and mRNA levels of *POSTN* in the LNCaP cells were significantly reduced (20). In another experimental model of human melanoma, the use of the function-blocking monoclonal antibody OC-20, specific for a YH motif localized in the second FAS1 domain of POSTN, which is a binding site for the integrins $\alpha v\beta 3$ and $\alpha v\beta 5$, was shown to inhibit tumor growth and to reduce tumor vascular density (21).

These results considered together, appear to suggest that beyond representing a putative marker of cells committed to tumor progression, *POSTN* could also represent a potential target for therapeutic interventions. As shown by our experiment and by those mentioned above, *POSTN* silencing is a complex mechanism which could be achieved either through the interference with POSTN interaction proteins as it could be the case for BIC in our experiment, or through directly targeting its gene expression, as it was the case in the experiment reported by Sun *et al*. (20), or through directly blocking the overexpressed protein with monoclonal antibodies, as in the case of the experiment reported by Orecchia *et al*. (21).

In conclusion, although our results should be considered with caution, due to the intrinsic limits of our experimental model, it appears particularly relevant that ectopic overexpression of *POSTN* resulted in increased cell proliferation, which was impaired by exposure to BIC, either in the presence or in the absence of concurrent exposure to DHT. These results attest to the potential role of POSTN in promoting cancer cell growth. The effects of *POSTN* gene transfection on other functional aspects, beyond tumor proliferation, need further characterization.

## Conflicts of Interest

The Authors declare no conflicts of interest.

## Acknowledgements

This work was partially supported by grants from the Italian Health Ministry (grant number 2005 conv. 93; 2005 conv. 65); Italian Education, University and Research Ministry (grant number: 9806118184 and 2008YFRLC8-004); Liguria Region, Department of Health (grant number: 563/2009); Compagnia di San Paolo, Italy (grant number: 2009.1271).

## References

1 Siegel R, Naishadham D and Jemal A: Cancer statistics, 2012. CA Cancer J Clin *62*: 10-29, 2012.
2 Rosenberg MT, Froehner M, Albala D and Miner MM: Biology and natural history of prostate cancer and the role of chemoprevention. Int J Clin Pract *64*: 1746-1753, 2010.
3 Dall'era MA, Albertsen PC, Bangma C, Carroll PR, Carter HB, Cooperberg MR, Freedland SJ, Klotz LH, Parker C and Soloway MS: Active surveillance for prostate cancer: a systematic review of the literature. Eur Urol *62(6)*: 976-983, 2012.
4 Bickers B and Aukim-Hastie C: New molecular biomarkers for the prognosis and management of prostate cancer-the post PSA era. Anticancer Res *29*: 3289-3298, 2009.
5 Boccardo F, Rubagotti A, Carmignani G, Romagnoli A, Nicolò G, Barboro P, Parodi S, Patrone E and Balbi C: Nuclear matrix proteins changes in cancerous prostate tissues and their prognostic value in clinically localized prostate cancer. Prostate *55*: 259-264, 2003.
6 Ricci F, Rubagotti A, Zinoli L, Mangerini R, Nuzzo PV, Carmignani G, Simonato A, Barboro P, Balbi C and Boccardo F: Prognostic value of nuclear matrix protein expression in localized prostate cancer. J Cancer Res Clin Oncol *138*: 1379-1384, 2012.
7 Kudo Y, Siriwardena BS, Hatano H, Ogawa I and Takata T: Periostin: Novel diagnostic and therapeutic target for cancer. Histol Histopathol *22*: 1167-1174, 2007.
8 Ruan K, Bao S and Ouyang G: The multifaceted role of periostin in tumorigenesis. Cell Mol Life Sci *66*: 2219-2230, 2009.
9 Morra L and Moch H: Periostin expression and epithelial mesenchymal transition in cancer: A review and an update. Virchows Arch *459*: 465-475, 2011.
10 Castronovo V, Waltregny D, Kischel P, Roesli C, Elia G, Rybak JN and Neri D: A chemical proteomics approach for the identification of accessible antigens expressed in human kidney cancer. Mol Cell Proteomics *5*: 2083-2091, 2006.
11 Tsunoda T, Furusato B, Takashima Y, Ravulapalli S, Dobi A, Srivastava S, McLeod DG, Sesterhenn IA, Ornstein DK and Shirasawa S: The increased expression of periostin during early stages of prostate cancer and advanced stages of cancer stroma. Prostate *69*: 1398-1403, 2009.
12 Tischler V, Fritzsche FR, Wild PJ, Stephan C, Seifert HH, Riener MO, Hermanns T, Mortezavi A, Gerhardt J, Schraml P, Jung K, Moch H, Soltermann A and Kristiansen G: Periostin is up-regulated in high-grade and high-stage prostate cancer. BMC Cancer *10*: 273, 2010.
13 Nuzzo PV, Rubagotti A, Zinoli L, Ricci F, Salvi S, Boccardo S and Boccardo F: Prognostic value of periostin expression in human prostate cancer: Correlation with clinical pathological features and risk of biochemical relapse or death. BMC Cancer *12*: 625, 2012.
14 Iversen P, Tyrrell CJ, Kaisary AV, Anderson JB, Van Poppel H, Tammela TL, Chamberlain M, Carroll K and Melezinek I: Bicalutamide monotherapy compared with castration in patients with non metastatic locally advanced prostate cancer: 6.3 Years of follow-up. J Urol *164*: 1579-1582, 2000.
15 Boccardo F, Rubagotti A, Barichello M, Battaglia M, Carmignani G, Comeri G, Conti G, Cruciani G, Dammino S, Delliponti U, Ditonno P, Ferraris V, Lilliu S, Montefiore F, Portoghese F and Spano G: Bicalutamide monotherapy *versus* flutamide plus goserelin in prostate cancer patients: Results of an Italian Prostate Cancer Project study. J Clin Oncol *17*: 2027-2038, 1999.
16 Mangerini R, Argellati F, Pfeffer U and Boccardo F: Effects of bicalutamide and 4OH-tamoxifen on androgen-regulated gene expression in the LNCaP cell line. Anticancer Res *32*: 5323-5329, 2012.
17 Boccardo F, Medicina D, Galmozzi F, Emionite L and Lo Casto M: 4-OH tamoxifen does not interfere with bicalutamide inhibitory effects on human prostatic cancer cells *in vitro*. Anticancer Res *25*: 4277-4280, 2005.
18 Boudreau N and Bissell MJ: Extracellular matrix signaling: Integration of form and function in normal and malignant cells. Curr Opin in Cell Biol *10*: 640-646, 1999.
19 Knudsen BS and Miranti CK: The impact of cell adhesion changes on proliferation and survival during prostate cancer development and progression. J Cell Biochem *99*: 345-361, 2006.
20 Sun C, Zhao X, Xu K, Gong J, Liu W, Ding W, Gou Y, Xia G and Ding Q: Periostin: A promising target of therapeutical intervention for prostate cancer. J Transl Med *30*: 9:99, 2011.
21 Orecchia P, Conte R, Balza E, Castellani P, Borsi L, Zardi L, Mingari MC and Carnemolla B: Identification of a novel cell binding site of periostin involved in tumour growth. Eur J Cancer *47*: 2221-2229, 2011.

*Received January 4, 2013*
*Revised February 14, 2013*
*Accepted February 15, 2013*