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ORIGINAL PAPER

Activin A circulating levels in patients with bone metastasis from breast or prostate cancer

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Abstract Recent studies have highlighted that Activin A, a member of the transforming growth factor-β (TGF-β) superfamily, may be involved in the regulation of osteoblastic activity and in osteoclast differentiation. Therefore, we have investigated the clinical significance of its circulating levels in patients with bone metastasis. Activin A serum concentrations were determined, by a commercially available enzyme-linked immunosorbent assay kit, in 72 patients with breast cancer (BC) or prostatic cancer (PC) with (BM+) or without (BM–) bone metastases, in 15 female patients with age-related osteoporosis (OP), in 20 patients with benign prostatic hypertrophy (BPH) and in 48 registered healthy blood donors (HS) of both sex (25 female and 23 male). Activin A serum concentrations were significantly increased in BC or PC patients as compared to OP ($P < 0.0001$) or BPH ($P = 0.045$), respectively, or to sex matched HS ($P < 0.0001$). Additionally, these levels resulted more elevated in PC patients as compared to BC patients ($P = 0.032$). Interestingly, Activin A was significantly higher in BM+ patients than in BM– patients (BC, $P = 0.047$; PC, $P = 0.016$). In BC patients, a significant correlation was observed only between Activin A and number of bone metastases ($P = 0.0065$) while, in PC patients, Activin A levels were strongly correlated with the Gleason score ($P = 0.011$) or PSA levels ($P = 0.0001$) and, to a lessen extent, with the number of bone metastases ($P = 0.056$). Receiver operating characteristic curve (ROC) analysis showed a fair diagnostic accuracy of Activin A to discriminate between BM+ and BM– patients (BC: AUC = 0.71 ± 0.09, $P = 0.03$; PC: AUC = 0.73 ± 0.081, $P = 0.005$). These findings indicate that Activin A may be implicated in the pathogenesis of bone metastasis. Therefore, this cytokine may be considered a novel potential target for a more selective therapeutic approach in the treatment of skeletal metastasis and may be also useful as additional biochemical marker of metastatic bone disease.

Keywords Activin A · Benign prostatic hypertrophy · Bone metastasis · Breast cancer · Neoplasm · Osteoporosis · Prostate cancer · Transforming growth factor β · Tumor markers

Abbreviations

AUC	Area under the curve
BC	Breast cancer
BPH	Benign prostatic hypertrophy
BM+	Bone metastasis
BM–	No metastasis
ELISA	Enzyme-linked immunosorbent assay
HS	Healthy subjects
OP	Primary osteoporosis
PC	Prostate cancer

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PSA Prostate specific antigen
ROC Receiver operating characteristic curve
TGF-β Transforming growth factor-β

Introduction

Activin A is a member of the transforming growth factor-β (TGF-β) superfamily, which comprises a group of growth factors with similar structure but different functions [1–3]. It was originally identified as an endocrine derived regulator of pituitary follicular stimulating hormone (FSH) [1, 2]. However, subsequent studies have shown that this multifunctional cytokine is widely distributed in different cells and tissues and that it appears to be actively involved in the regulation of several important biological functions such as cell proliferation, apoptosis, differentiation, immune response, wound healing, embryogenesis, endocrine functions, tissue metabolism and homeostasis [1–8]. The biological effects of Activin A, are mediated through two transmembrane serine/threonine receptor kinases namely, ActRI and ActRII and may be inhibited by a number of Activin binding proteins [1, 2, 9]. Interestingly, a consistent number of experimental and clinical observations have reported that many human cancers present altered expression levels of Activin A and/or altered levels of its receptors and/or that of its specific inhibitors and that these alterations may be associated with a more malignant tumor phenotype [3, 4, 10–20]. Furthermore, recent findings have shown that, Activin A may play an active role in the modulation of osteoblastic activity and in osteoclast differentiation during bone remodeling processes associated to a number of physiological or pathological conditions [1, 5–8, 10, 21, 22]. Therefore, it has been hypothesized that this growth factor may play also a role in bone metastasis formation [10]. On the basis of these considerations we have undertaken some studies to assess the clinical significance of the circulating levels of Activin A in patients with breast (BC) or prostate cancer (PC) as these tumors preferentially metastasize to the bone and induce the formation of osteolytic and/or osteoblastic metastasis, respectively [23].

Patients and methods

The study included 48 registered healthy blood donors (HS) of both sex (25 female and 23 male) who served as control group, 15 female patients with age-related osteoporosis (OP) (mean age 74.1 ± 10.5 years), 20 patients with histological confirmed benign prostate hypertrophy (BPH) (mean age 64.0 ± 6.7 years) and 72 patients with breast cancer (mean age 57.7 ± 10.2 years) or prostate cancer (mean age 71.03 ± 8.1 years) with confined disease (BM–) or skeletal metastasis only (BM+). The main characteristics of cancer patients are reported in Table 2. Bone metastases were diagnosed by skeletal scintigraphy and/or skeleton X-ray or, where required, by magnetic resonance imaging. None of the metastatic patients had documented extraskeletal lesions. The study was approved by local ethical committee and carried out in accordance with the Declaration of Helsinki [24].

Activin A assay

Blood samples from cancer patients or patients with OP or BPH were obtained before starting any therapy. The samples were drawn into polycarbonate tubes, allowed to clot at room temperature and then centrifuged at 3,500 rpm for 15 min (Hereus Omnifuge 2.0 RS, Hereus Sepatech). Serum aliquots were stored at –80°C until assays. Activin A serum concentrations were determined by a commercially available two-step sandwich enzyme-linked immunosorbent assay (ELISA) kit according the manufacturer's instructions (Activin A Assay kit, Serotec, Oxford Bio-Innovation Ltd, UK). The reported detection limit was < 78 pg/ml. According to the manufacturer the assay has no detectable cross reactivity with Inhibin A, Follistatin, Activin B, or Inhibin B while a small cross reaction (1–5%) may be observed with Activin AB.

Serum tumor markers

The serum levels of tumor marker Ca15.3 were additionally measured in BC and OP patients while PSA levels were determined in PC and BPH patients. Both markers were determined by commercially available immunoluminometric assays kits (DiaSorin, Germany). The reference values and the detection limits reported by the manufacturer were 30 U/ml and < 0.3U/ml for Ca15.3 and 3.2 ng/ml and < 0.009 ng/ml for PSA, respectively.

Statistical analysis

Statistical analysis was performed by using the Medcalc 7.4 statistical software package (Medcalc Mariakerke, Belgium) (MEDCALC version 7.4 Copyright 1993–2004, Frank Schoonjans http://www.medcalc.be). Data were tested for normal distribution by the

Kolmogorov–Smirnov test. Because of their uneven distribution the statistical analysis was performed, where required, by the non-parametric Mann–Whitney *U* test, the Kruskall–Wallis test of variance and the Spearman rank correlation test (r_s). The receiver operating characteristic curve (ROC) was generated to assess the sensitivity and the specificity of serum Activin A for detection of bone metastasis.

Results

Activin A serum levels in HS, OP, BPH, BC and PC patients are shown in Table 1. No gender related difference in the serum concentrations of Activin A were observed in HS. The circulating levels of this growth factor were significantly increased in BC or BC patients as compared to OP patients ($P < 0.0001$) or BPH patients ($P = 0.045$), respectively, or to sex matched HS ($P < 0.0001$). Significant differences were also highlighted between BPH patients and HS ($P = 0.0065$) (Table 1). Interestingly, Activin A serum levels were more elevated in PC patients as compared to BC patients ($P = 0.032$) (Table 1) and were also significantly higher in BC or PC patients with bone metastasis as compared to patients with confined disease (BC, $P = 0.047$; PC, $P = 0.016$) (Fig. 1). Table 2 shows the distribution of serum Activin A in BC or PC patients according to some clinicobiological parameters. In BC patients, Activin A concentrations significantly correlated only with the number of bone metastases ($P = 0.0065$) while, in PC patients Activin A levels were significantly associated with the Gleason score ($P = 0.011$) or PSA levels ($P = 0.0001$) and weakly with the number of bone lesions ($P = 0.056$) (Table 2). ROC curve analysis was performed to assess the effectiveness of serum Activin A to discriminate between cancer patients with bone metastases from those without bone metastases. At the cut-off level determined by ROC curve, Activin A showed a fair diagnostic accuracy to discriminate between BM+ and BM– patients (BC: AUC = 0.71 ± 0.09, 95% CI: 0.52–0.85, $P = 0.03$; PC: AUC = 0.73 ± 0.081, 95% CI: 0.56–0.86, $P = 0.005$) (Fig. 2). However, in our series of patients, serum markers Ca15.3 and PSA showed a better diagnostic performance (Table 3). In BC patients, the combination of Activin A with Ca15.3, at the cut-off levels considered in this study, resulted in a lower sensitivity (50%) and in a higher specificity (100%) if compared to Ca15.3 alone (Table 3) while, in PC patients, Activin A did not substantially affect the good diagnostic performance of PSA alone in detecting BM+ patients (Table 3).

Table 1 Activin A serum concentrations (ng/ml) in healthy subjects and in patients with non-malignant or malignant diseases

	No of subjects	Median	Range	Mean ± SD
Healthy subjects	48	0.41	0.10–0.82	0.43 ± 0.17
Female	25	0.42	0.22–0.60	0.43 ± 0.12
Male	23	0.40	0.10–0.82	0.44 ± 0.21
Primary osteoporosis	15	0.38	0.22–0.59	0.37 ± 0.09
Benign prostatic hyperplasia	20	0.68	0.37–1.34	0.67 ± 0.26[a]
Breast cancer	33	0.60	0.33–4.79	0.92 ± 0.94[b,c]
Prostate cancer	39	0.80	0.23–3.33	1.03 ± 0.70[b,d,e]

Data analysis was computed by the Mann–Whitney *U* test; [a]$P = 0.0002$ and [b]$P < 0.0001$ vs HS; [c]$P < 0.0001$ vs OP; [d]$P = 0.045$ vs BPH; [e]$P = 0.032$ vs BC

Conclusions

Several studies suggest that Activin A may have a role in tumor progression [2, 3, 10–25]. The specific

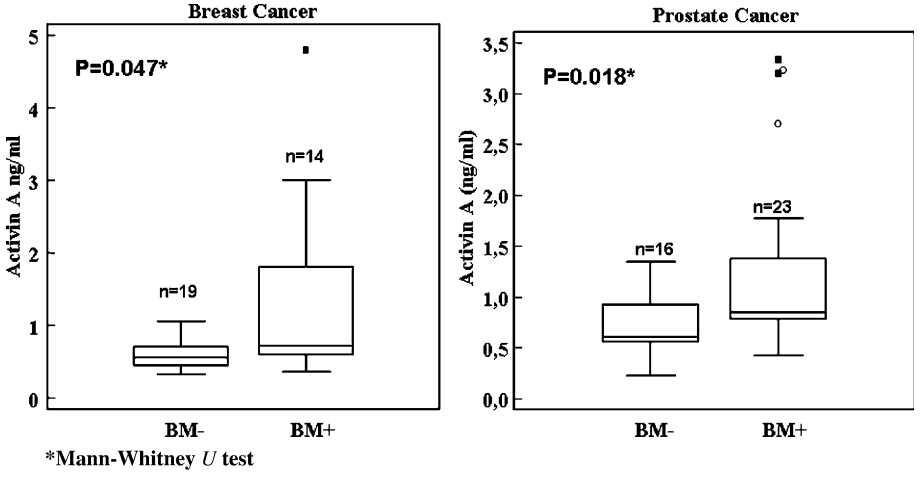

Fig. 1 Activin A serum distribution in breast or prostate cancer patients with (BM+) or without (BM–) bone metastases

Table 2 Activin A distribution in patients with breast or prostate cancer according to some clinicobiological parameters

	No. pts (%)	Activin A (ng/ml)	*P* value
Breast cancer			
Tumor size			
T1	8 (24.2%)	0.56	
T2	18 (54.5)	0.66	0.099(N.S.)[a]
T3-T4	7 (21.8)	0.93	
Tumor grade			
G1	7 (21.8)	0.53	
G2	16 (48.5)	0.60	0.081 (N.S.)[a]
G3	10 (30.3)	0.88	
Estrogen receptors			
Negative	6 (20)	0.56	
Positive	24 (80)	0.66	0.83 (N.S.)[b]
Progesteron receptors			
Negative	23 (79.3)	0.56	
Positive	6 (20.7)	0.83	0.12 (N.S.)[b]
Ca15.3			
< 30 U/ml	21 (65.6%)	0.55	
> 30 U/ml	11 (35.4)	0.72	0.17 (N.S.)[b]
Number of metastases			
M0	19 (57.6)	0.55	
M1-3	7 (21.2)	0.60	0.0065[a]
M > 3	7 (21.2)	1.8	
Prostate cancer			
Tumor size	n.d.[d]		
Gleason score			
2–4	8 (20.5)	0.57	
5–7	22 (56.4)	0.83	0.011[a]
8–10	9 (23.1)	1.40	
PSA			
≤ 4 ng/ml	10 (27.0)	0.56	
> 4 ng/ml < 10 ng/ml	9 (24.3)	0.63	< 0.0001[a]
> 10 ng/ml	18 (48.7)	0.89	
Number of metastases			
M0	16 (41.0)	0.59	
M1-3	4 (10.3)	0.81	0.056[a] (N.S.)
M > 3	1 9 (48.7)	0.94	

N.S., not significant; n.d., not determined; [a]Kruskall–Wallis test; [b]Mann–Whitney *U* test

mechanisms by which this growth factor may facilitate this phenomenon have still not fully understood. In vitro studies have shown that Activin A can stimulate or inhibit tumor cell proliferation according to the tumor type [2–4]. Therefore, in tumor in which Activin A has a growth inhibitory activity, in order to proliferate the tumor cell must acquire resistance to this growth factor. Conversely, in tumor in which Activin A stimulate, tumor cell proliferation, a sustained Activin A signaling would promote tumor growth [2–4]. Some experimental studies, have indicated that these effects may occur in tumor cells following the disregulation of Activin signaling pathway caused by alterations in the expression levels of this cytokine and/or in that of its receptors and/or inhibitors or following loss or mutations of specific genes codifying for its receptors [2, 3, 10, 11–18, 21, 27–29]. The possible correlation between these mechanisms and tumor progression is corroborated by a number experimental and clinical observations which have highlighted that these alterations may be present in several neoplasms including breast and prostate cancer and are associated with more aggressive forms of these neoplastic diseases [2–4, 10–19, 25–29]. Furthermore, in vitro studies indicate that this growth factor, in addition to its effects on cell proliferation, apoptosis and malignant transformation, may also facilitate the homing and spread of tumor cells in several organs including the bone [3, 7, 10, 21, 30, 31]. Our results appear to support this hypothesis. In fact, it has been shown that (i) Activin A circulating levels were significantly increased in cancer patients as compared to healthy subjects or patients with benign diseases; (ii) BC or PC patients with bone metastases had serum concentrations of Activin A significantly higher than those measured in patients with confined diseases; (iii) in both BC and PC patients, the serum levels of this growth factor were positively associated with the number of skeletal metastases. Furthermore, mean Activin A serum concentrations were significantly more elevated in PC patients than in BC patients. This latter phenomenon further indicate, as reported in other studies, that Activin A may be actively involved in the modulation of the osteoblastic activity which, in the case of prostate cancer, is predominant and may account for osteoblastic reactions induced by tumor cells [8, 12, 23, 31, 32]. In addition, our results have highlighted that, in PC patients Activin A levels significantly correlated with PSA serum concentrations. These data may fit well with the results from in vitro studies of Fujii et al. [33] which have shown that Activin A upregulates PSA gene expression and increases the secretion of this serine proteinase in LNCaP cells. On the other hand, the significant correlation of Activin A with the Gleason score further confirm previous immunohistochemical observations of Cardillo et al. [34] on the positive correlation between Activin A overexpression and more aggressive forms of this tumor. Finally, ROC curve analysis showed a fair diagnostic accuracy of Activin A to detect patients with bone metastasis. However, in our series of patients, its diagnostic performance did not result significantly superior to those of PSA or Ca15.3. Nevertheless, the combination of Activin A with Ca15.3, at the cut-off level considered in this study, correctly identified 100% of BC patients without bone

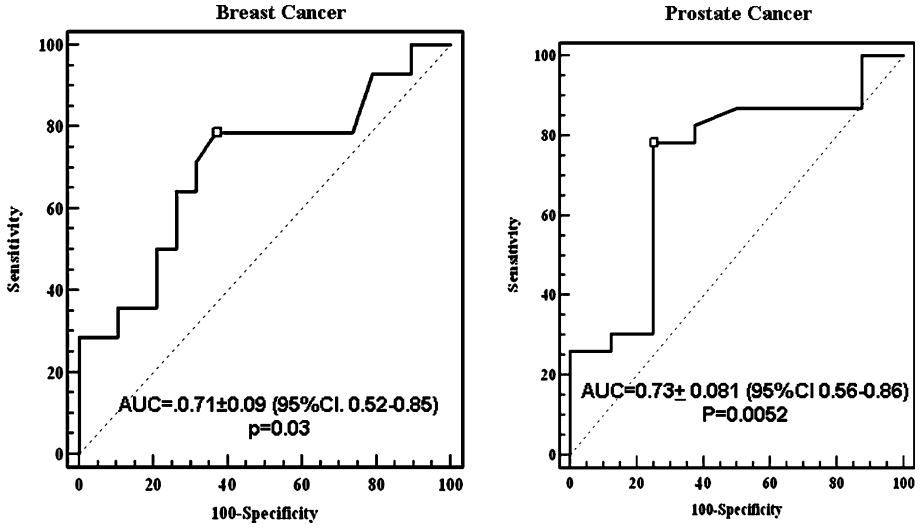

Fig. 2 Receiver operating characteristic (ROC) curve for Activin A to discriminate between BC or PC patients with and without bone metastases

Table 3 Sensitivity and specificity of Activin A, Ca15.3 or PSA alone or in combination in the detection of bone metaseses of breast or prostate cancer

	Cut-off value[a]	Sensitivity (95% CI)	Specificity (95% CI)
Breast cancer			
Activin A	> 0.57 ng/ml	78.6 (45.1–79.6)	63.2 (38.4–83.6)
Ca15.3	> 30.1 U/ml	64.3 (35.2–87.1)	94.7 (73.9–99.1)
Activin A + Ca15.3		50.0 (23.1–76.9)	100 (82.2–100)
Prostate cancer			
Activin A	> 0.74 ng/ml	78.3 (56.3–95.2)	75.0 (47.6–92.6)
PSA	> 16.9 ng/ml	82.6 (61.2–94.9)	100 (78.0–100)
Activin A + PSA		78.3 (56.3–95.2)	100 (79.2–100)

[a] Cut off values were determined by ROC curve analysis

metastases while, the combination of Activin A and PSA did non-substantially affect the diagnostic performance of this latter tumor marker. In conclusion, the present investigations suggest that Activin A may be implicated in bone metastasis formation from breast or prostate cancer. Therefore, this growth factor may be considered a novel potential target for a more effective therapeutic approach in the treatment of bone metastasis. On the other hand, further studies with a wider number of patients may better define the clinical role of Activin A as additional biochemical marker of metastatic bone disease.

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