Meta Gene

Volume 17, September 2018, Pages 124-127

# Role of osteopontin and its rs11730582 gene polymorphism in breast cancer

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https://doi.org/10.1016/j.mgene.2018.05.006



## Abstract

### Background

Osteopontin (OPN) is an extracellular matrix protein of the integrin family that is involved in several biological pathways. Genetic polymorphisms in OPN gene has been found associated with many types of cancer.

### Aims

In this investigation, we aimed to study the possible role of OPN and its rs11730582 C/T single nucleotide polymorphism (SNP) in patients with breast cancer.

### Methods

OPN plasma levels were measured by ELIZA and OPN rs11730582 was genotyped by Taqman genotyping assay in 60 breast cancer patients and 60 healthy controls.

### Results

Obtained results revealed a significant increase in plasma OPN levels in the patients compared to the controls (mean±SD was 194.8±43 in patients versus 80.6±34.7 in controls, *P*<0.0001). OPN plasma level was affected by rs11730582 genotype and the hormones receptors status of the tumor (*P*<0.05), while, no effect of the stage, grade or histological type of the tumor on OPN plasma level was found. The distribution of OPN rs11730582 genotypes differed significantly in the patients compared to controls, TT-genotype was significantly higher in breast cancer patients (*P*<0.05) and was associated with elevated OPN levels. In addition, rs11730582 genotypes distribution was associated with tumor grade and hormones receptors status. Multivariate analysis revealed that plasma OPN levels and OPN rs11730582 C/T SNP genotypes distribution were associated with breast cancer after adjustment of other variables.

Conclusion

In conclusion, OPN and its rs11730582 SNP were implicated in breast cancer in our study.

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Keywords

Breast cancer; Gene polymorphism; Osteopontin

## 1. Introduction

Breast cancer is the most common cancer in women. Breast cancer has been found to occur in about 15% of women during their lifetime (Xiaoxian et al., 2017). Genome-wide association studies (GWAS) have identified several SNPs that contribute in breast cancer risk (Bayraktar et al., 2013).

Osteopontin (OPN) is an acidic glycoprotein of the small integrin binding N-linked glycoprotein family that is clustered on human chromosome 4, location 4q22.1 (Fisher et al., 2001). It is considered as an inflammatory cytokine that promotes cellular activation, migration and chemotaxis and it also possesses angiogenic properties (Sodek et al., 2000). OPN is expressed at increased levels by the tumor

cells and has a diagnostic and prognostic potential in many types of cancer (Shevde and Samant, 2014). It is involved in tumor progression, and metastasis, so representing an attracting target for cancer therapy (Castello et al., 2017).

OPN rs11730582 (-443C/T) is the most SNP of OPN gene that has been studied. It is located in the promotor region of OPN gene and has been found to play a role in various types of cancer (Briones-Orta et al., 2017). Polymorphic sites in the promoter region may influence transcription factor binding and gene expression. They can also affect RNA stability and lead to altered protein levels in the cancer cells (Ramchandani and Weber, 2013).

In this investigation, we aimed to study the possible role of OPN and its rs11730582 (−443C/T) gene polymorphism in Egyptian patients with breast cancer.

## 2. Subjects and methods

### 2.1. Subjects

The study was approved by Sohag Faculty of Medicine Ethics committee and an informed written consent was obtained from all the participants. The study was carried out on 60 female patients with different stages of breast cancer recruited from the department of Surgery, Faculty of Medicine, Sohag University from the period of July 2016 to July 2017. The sample size of the study group was calculated using a program at (www.openepi.com/SampleSize/ SSCC.htm), adjusted to achieve 80% power and 5% confidence of significance (type I error). The demographic data of patients including age, parity, menopausal status, stage, estrogen receptors (ER) status, progesterone receptors (PR) status, grade and histopathological type of cancer were collected from their medical records. Exclusion criteria were; Patients with malignancies elsewhere in the body, Patients with chronic medical diseases (cardiac, hepatic or renal and diabetes), those currently pregnant or breast feeding and Patients with distant metastasis. Sixty healthy women with no history of medical problems and matched to the patients regarding age, parity and menopausal status were included as controls.

### 2.2. Blood collection and DNA extraction

About 5ml venous blood sample from each participant was collected in ethylene-diamine-tetraacetic acid (EDTA) tubes under aseptic conditions and centrifuged at 3000×*g* for 15min. Plasma was separated in microcentrifuge tubes, stored at −20 for OPN level assay and the mononuclear cell layer was immediately used for DNA

extraction. DNA extraction was done using spin column based Blood-Animal-Plant DNA Preparation Kit supplied by Applied Biosystems according to enclosed instructions.

### 2.3. Assay of OPN plasma level

The plasma level of OPN was measured by an enzyme–linked immunosorbent assay technique using human OPN ELISA Kit supplied by SinoGeneClon Biotech Co., Ltd., Catalog No: SG-10445 according to the manufacturer's instructions, and results were determined by the Stat fax 2600 microplate reader (Awareness Technologies, Palm City, USA).

### 2.4. Genotyping

OPN rs11730582 C/T SNP genotyping was done using TaqMan SNP genotyping Assay (ID C___1840808_10). PCR was done using StepOne real time PCR system (Applied Biosystem, Ca, USA). The amplification was done using 25µl reaction volume containing 12.5µl TaqMan® Genotyping Master Mix, 1.25µl specific TaqMan® SNP genotyping assay (containing Sequence-specific forward and reverse primers to amplify the polymorphic sequence of interest and two TaqMan® MGB probes; One probe labeled with VIC® dye to detect the allele 1 sequence and another probe labeled with FAM™ dye to detect the allele 2 sequence) and 5µl (20ng) of genomic DNA. The reaction mixture was held at 95°C for 10min for AmpliTaq Gold enzyme activation, followed by 40 amplification cycles. Each cycle consisted of denaturation at 95°C for 15s, primer annealing and extension at 60°C for 60s. The study data were analyzed by The TaqMan® Genotyper™ Software.

### 2.5. Statistical analysis

Quantitative data were represented as mean, standard deviation, median and range. Genotype distribution was tested for deviation from Hardy–Weinberg equilibrium (HWE) by $x^2$ analysis. Data were analyzed using student *t*-test to compare means of two groups and ANOVA for comparison of the means of three groups or more. Linear regression between the variables and multivariate analysis using partial correlation test were done. Qualitative data were presented as number and percentage and compared using either Chi-square test or fisher exact test. Graphs were produced by using GraphPad Prism or SPSS programs. *P*-value was considered significant if it was <0.05.

## 3. Results

The characteristics of the participants in the study were shown in Table 1 and characteristics of the breast cancer patients were shown in Table 2. The results of the investigation revealed non-significant differences between the patients and the controls regarding age, parity or menopausal status ($P>0.05$), Table 1. However, plasma OPN levels and OPN rs11730582 C/T SNP genotypes distribution showed significant differences between the patients and the controls. Mean±SD plasma OPN level was 194.8±43 in patients versus 80.6±34.7 in controls ($P<0.0001$), Fig. 1. The genotypes CC, CT and TT in the patients and controls were 9 (15%), 22 (36.67%) and 29 (48.33%) respectively in the patients and 21 (35%), 27 (45%) and 12 (20%) respectively in the controls ($P=0.045$), Fig. 2. TT genotype and T-allele were significantly higher in the patients compared to the controls. Patients with TT genotype had significantly higher plasma OPN level than those with CC or CT genotypes, Fig. 3. Plasma OPN levels were significantly higher in patients with ER −ve and PR −ve r tumors than in those with +ve hormones receptors tumors. However its levels were not associated with tumor grade or the stage of the disease. Regarding OPN rs11730582 C/T SNP genotypes distribution, TT-genotype was associated with the presence of ER −ve and PR −ve tumors and with grade 3 tumors. Multivariate analysis revealed that plasma OPN levels and OPN rs11730582 C/T SNP genotypes distribution were associated with breast cancer after adjustment of other variables. Odd ratios (95% CI) for the association between T-allele and TT-genotype in all patients versus the controls using the dominant and the recessive genetic models were shown in Table 3. Receiver operating curve (ROC) of OPN showed that OPN level as a strong predicting factor for breast cancer (area under curve=0.959, $P<0.0001$), Fig. 4.

Table 1. Characteristics of the participants.

| Variable | Cases (n=60) | Controls (n=60) | *P*-value |
|---|---|---|---|
| **Age (years)** | | | |
| **Mean±SE** | 57.42±1.26 | 55.05±1.80 | 0.33 |
| **Median (range)** | 57 (39–75) | 55 (40–70) | |
| **Parity** | | | |
| **Mean±SE** | 4.32±0.23 | 4.1±0.19 | 0.42 |

| | | | |
|---|---|---|---|
| **Median (range)** | 4 (0–8) | 4 (3–6) | |
| **Menopausal status** | | | |
| **Pre-menopause** | 23 (38.33%) | 21 (35%) | 0.79 |
| **Post-menopause** | 37 (61.67%) | 39 (65%) | |

Table 2. Characteristics of breast cancer.

| Variable | Number (%) |
|---|---|
| **Histological type** | |
| **Ductal carcinoma** | 39 (65.00%) |
| **Lobular carcinoma** | 21 (35.00%) |
| **Stage** | 15 (25.00%) |
| **Stage 1** | 22 (36.67%) |
| **Stage 2** | 23 (38.33%) |
| **Grade** | |
| **Grade 1** | 10 (16.67%) |
| **Grade 2** | 23 (38.33%) |
| **Grade 3** | 27 (45.00%) |
| **Estrogen receptors (ER)** | |
| **Negative** | 30 (50.00%) |
| **Positive** | 30 (50.00%) |
| **Progesterone receptors (PR)** | |
| **Negative** | 39 (65.00%) |
| **Positive** | 21 (35.00%) |

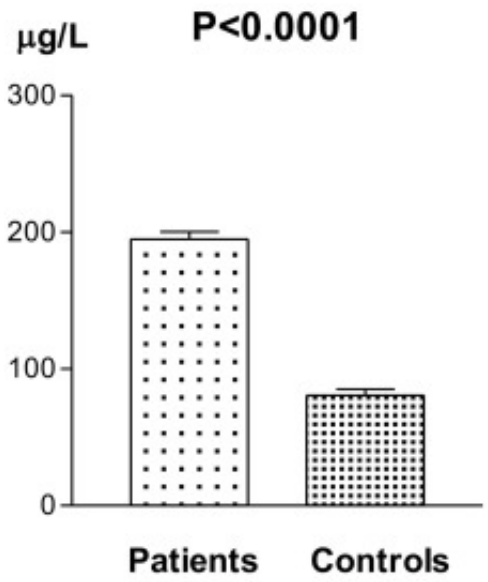


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Fig. 1. OPN level (µg/L) in patients and in controls.

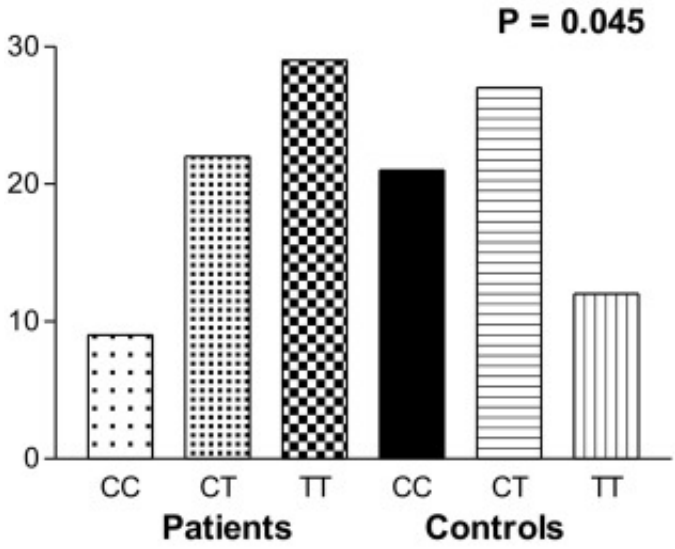


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Fig. 2. OPN rs11730582 C/T genotypes distribution (represented as numbers) in the participants.

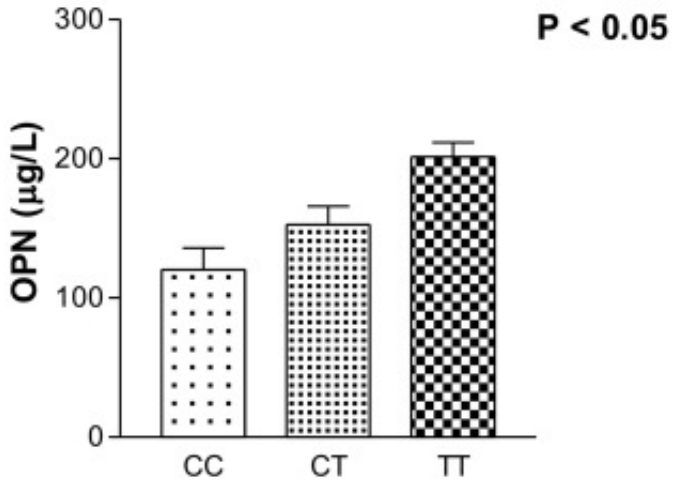


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Fig. 3. OPN level (mean±SD) in the patients according to genotype.

Table 3. ORs (95% CI) in all patients versus the controls using the dominant and the recessive genetic models.

| Model | OR (95% CI), *P*-value |
|---|---|
| **Dominant model (allele T increase the risk)** | 3.05 (1.26t–7.4), *P*=0.0135 |
| **Recessive model (TT-genotype increase the risk)** | 2.26 (0.97–5.25), *P*=0.048 |

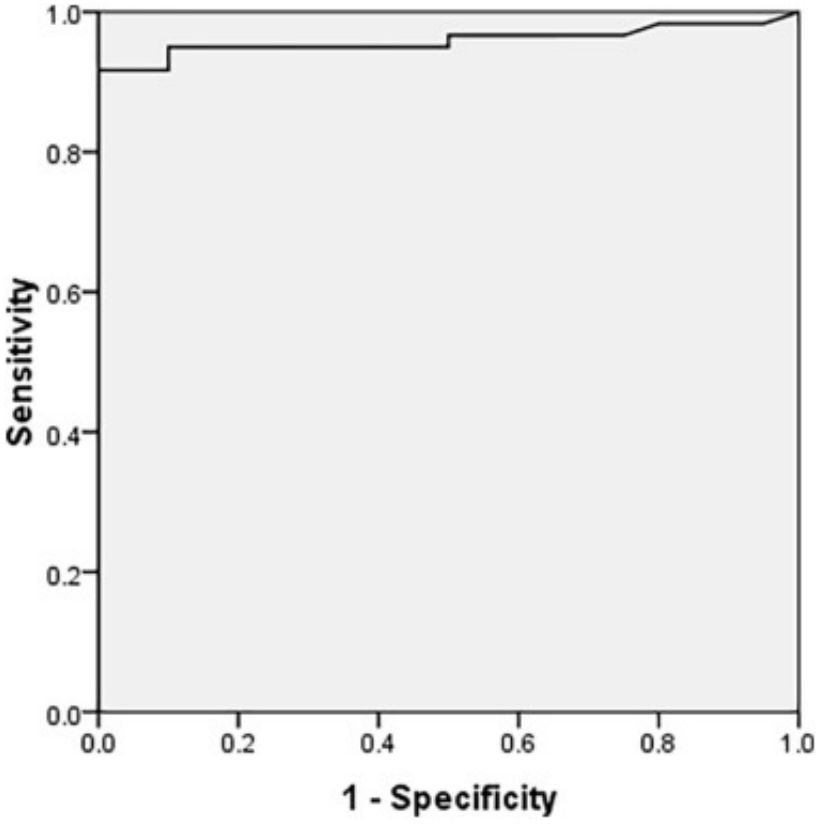


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Fig. 4. Receiver operating curve (ROC) of OPN.

## 4. Discussion

The investigation revealed significant differences in plasma OPN and OPN rs11730582 (−443C/T) SNP between breast cancer patients and healthy controls. Plasma OPN was significantly higher and TT-genotype and T-allele were more prevalent in the patients compared to the controls. TT- genotype was more common in patients with ER and PR negative tumors than in those with ER and PR positive tumors.

OPN plays a key role in cancer progression by enhancing proliferation, survival, motility, and invasion of tumor cells in many cancer types including; breast cancer, hepatic carcinoma, prostate cancer, colorectal cancer, lung cancer, and melanoma (Cook et al., 2005; Bandopadhyay et al., 2014; Irby et al., 2004; Zhou et al., 2005; Chambers et al., 1996; Thalmann et al., 1999; Gotoh et al., 2002). Overexpression of OPN has been detected at the tumor sites and in the blood of patients, and its levels were found correlated with tumor stage and aggressiveness, indicating that

OPN can be a diagnostic and prognostic biomarker for several cancers (Irby et al., 2004). The expression of OPN in breast cancer was reported to correlate with the aggressiveness of cancer, and knocking down endogenous OPN expression reduced invasive behavior and suppressed tumor growth in immunocompromised mice (Kale et al., 2014). OPN was reported to mediate increase in migration, motility, and invasion of tumor cells. This could be attributed to the enhanced expression of integrins and CD44 cell surface receptors and to the increase in metabolic activity (Rodrigues et al., 2007). An enhanced expression of OPN was found in breast cancer tissue and it was concomitant with loss of Merlin expression at the protein level. Merlin is a cytoskeleton membrane protein that has been demonstrated to function as a tumor suppressor in breast cancer. OPN was found to initiate serine/threonine kinase (Akt)-mediated phosphorylation and degradation of Merlin in breast cancer cells (Morrow et al., 2011). In addition, OPN enhances the expression of vascular endothelial growth factor (VEGF) through AKT and extracellular signal-regulated kinase (ERK) phosphorylation (Dai et al., 2009). OPN can be upregulated by fibroblast growth factor 2 in endothelial cells in vitro and in vivo, leading to the recruitment of proangiogenic monocytes to the tumor microenvironment (Leali et al., 2003). It has been shown that thrombin could cleave OPN into two fragments, OPN-N and OPN-C, that has a stronger angiogenic potential in vitro than full-length OPN (Senger et al., 1996). Senger et al. showed that VEGF induces OPN in endothelial cells and OPN cleavage by thrombin resulting in OPN fragments that were more strongly chemotactic for endothelial cells and had the ability to promote angiogenesis than intact OPN (Senger et al., 2002).

The expression of OPN is influenced by genetic polymorphisms in its promoter. rs11730582 polymorphism was studied in many types of cancer and −443 TT genotype was found correlated with increased expression of OPN in breast cancer. Additionally, −443 TT was associated with tumor grade, and T- allele was more common in high grade tumors. It is also more common among patients with high OPN levels compared with those with lower OPN levels. Also, T−allele was more common in estrogen receptors negative and progesterone receptors negative cancers (Ramchandani and Weber, 2013). However, in other tumors, including acute myeloid leukemia, glioma, and papillary thyroid cancer −443CC genotype was associated with higher expression of OPN and increased cancer risk (Zhang et al., 2015, Shen et al., 2014, Mu et al., 2013). These opposing results can be explained as the proto-oncogene c-Myb mediates induction of OPN expression

levels from the C allele in some tumors, whereas in other malignancies, there is an unidentified transcription factor that might activate transcription of OPN from the T allele (Schultz et al., 2009). An in- silico analysis was performed (Briones-Orta et al., 2017) to predict the DNA-binding sites for transcription factors to the −443 C or T carrier sequences using the TRANSFAC v.6.4 (Farre et al., 2003; Messeguer et al., 2002). The analysis revealed that −443T allele sequence might have a predicted binding site for the signal transducer and activator of transcription protein (STAT6) that was not predicted in the C allele sequence. In breast cancer, the −443T allele carrier phenotype is associated with the worst prognosis, and this could be related to both, the presence of the T allele which has the putative binding site for STAT6 protein (Briones-Orta et al., 2017). The constitutive activation of STAT6 was reported in primary breast tumors and it might play a dual role as both tumor suppressor and tumor promoter (Bruns and Kaplan, 2006, Gooch et al., 2002). In conclusion, the study revealed that OPN and its rs11730582 gene polymorphism might have a role in the pathogenesis and prognosis of breast cancer in our community and this may help in the development of new therapeutic strategies against the disease. As many treatment strategies focus on the signaling pathways of tumor cells, therefore, OPN can be regarded as a target for breast cancer therapy.



## References

Bandopadhyay et al., 2014 M. Bandopadhyay, A. Bulbule, R. Butti, *et al.*
**Osteopontin as a therapeutic target for cancer**
Expert Opin. Ther. Targets, 18 (2014), pp. 883-895
CrossRef View Record in Scopus

Bayraktar et al., 2013 S. Bayraktar, P.A. Thompson, S.-Y. Yoo, *et al.*
**The relationship between eight GWAS-identified single-nucleotide polymorphisms and primary breast cancer outcomes**
Oncologist, 18 (2013), pp. 493-500
CrossRef View Record in Scopus

Briones-Orta et al., 2017 M.A. Briones-Orta, S.E. Avendaño-Vázquez, D.I. Aparicio-Bautista, J.D. Coombes, G.F. Weber, W.K. Syn
**Osteopontin splice variants and polymorphisms in cancer progression and prognosis**

Biochim. Biophys. Acta, 1868 (2017), pp. 93-108.A, 10.1016/j.bbcan.2017.02.005 (Epub 2017 Feb 28)
Article Download PDF View Record in Scopus

Bruns and Kaplan, 2006 H.A. Bruns, M.H. Kaplan
**The role of constitutively active Stat6 in leukemia and lymphoma**
Crit. Rev. Oncol. Hematol., 57 (2006), pp. 245-253
Article Download PDF View Record in Scopus

Castello et al., 2017 L.M. Castello, D. Raineri, L. Salmi, *et al.*
**Osteopontin at the crossroads of inflammation and tumor progression**
Mediat. Inflamm., 4049098 (2017), 10.1155/2017/4049098

Chambers et al., 1996 A.F. Chambers, S.M. Wilson, N. Kerkvliet, *et al.*
**Osteopontin expression in lung cancer**
Lung Cancer, 15 (1996), pp. 311-323
Article Download PDF View Record in Scopus

Cook et al., 2005 A.C. Cook, A.B. Tuck, S. McCarthy, *et al.*
**Osteopontin induces multiple changes in gene expression that reflect the six "hallmarks of cancer" in a model of breast cancer progression**
Mol. Carcinog., 43 (2005), pp. 225-236
CrossRef View Record in Scopus

Dai et al., 2009 J. Dai, L. Peng, K. Fan, *et al.*
**Osteopontin induces angiogenesis through activation of PI3K/AKT and ERK1/2 in endothelial cells**
Oncogene, 28 (2009), pp. 3412-3422
CrossRef View Record in Scopus

Farre et al., 2003 D. Farre, R. Roset, M. Huerta, *et al.*
**Identification of patterns in biological sequences at the ALGGEN server: PROMO and MALGEN**
Nucleic Acids Res., 31 (2003), pp. 3651-3653
CrossRef View Record in Scopus

Fisher et al., 2001 L.W. Fisher, D.A. Torchia, B. Fohr, *et al.*
**Flexible structures of SIBLING proteins, bone sialoprotein, and osteopontin**
Biochem. Biophys. Res. Commun., 280 (2001), pp. 460-465
Article Download PDF View Record in Scopus

Gooch et al., 2002 J.L. Gooch, B. Christy, D. Yee

**STAT6 mediates interleukin-4 growth inhibition in human breast cancer cells**
Neoplasia, 4 (2002), pp. 324-331
Article Download PDF CrossRef View Record in Scopus

Gotoh et al., 2002 M. Gotoh, M. Sakamoto, K. Kanetaka, *et al.*
**Overexpression of osteopontin in hepatocellular carcinoma**
Pathol. Int., 52 (2002), pp. 19-24
CrossRef View Record in Scopus

Irby et al., 2004 R.B. Irby, S.M. McCarthy, T.J. Yeatman
**Osteopontin regulates multiple functions contributing to human colon cancer development and progression**
Clin. Exp. Metastasis, 21 (2004), pp. 515-523
CrossRef View Record in Scopus

Kale et al., 2014 S. Kale, R. Raja, D. Thorat, *et al.*
**Osteopontin signaling upregulates cyclooxygenase-2 expression in tumor-associated macrophages leading to enhanced angiogenesis and melanoma growth via α9β1 integrin**
Oncogene, 33 (2014), pp. 2295-2306
CrossRef View Record in Scopus

Leali et al., 2003 D. Leali, P. Dell'Era, H. Stabile, *et al.*
**Osteopontin (Eta-1) and fibroblast growth factor-2 cross-talk in angiogenesis**
J. Immunol., 171 (2003), pp. 1085-1093
CrossRef View Record in Scopus

Messeguer et al., 2002 X. Messeguer, R. Escudero, D. Farre, *et al.*
**PROMO: detection of known transcription regulatory elements using species-tailored searches**
Bioinformatics, 18 (2002), pp. 333-334
CrossRef View Record in Scopus

Morrow et al., 2011 K.A. Morrow, S. Das, B.J. Metge, *et al.*
**Loss of tumor suppressor Merlin in advanced breast cancer is due to post-translational regulation**
J. Biol. Chem., 286 (2011), pp. 40376-40385, 10.1074/jbc.M111.250035
CrossRef View Record in Scopus

Mu et al., 2013 G. Mu, H. Wang, Z. Cai, *et al.*
**OPN-443C>T genetic polymorphism and tumor OPN expression are associated with the risk and clinical features of papillary thyroid cancer in a Chinese cohort**

Cell. Physiol. Biochem., 32 (2013), pp. 171-179
CrossRef View Record in Scopus

Ramchandani and Weber, 2013 D. Ramchandani, G.F. Weber
**An osteopontin promoter polymorphism is associated with aggressiveness in breast cancer**
Oncol. Rep., 30 (2013), pp. 1860-1868
CrossRef View Record in Scopus

Rodrigues et al., 2007 Lígia R. Rodrigues, José A. Teixeira, Fernando L. Schmitt, Marie Paulsson, Helena Lindmark-Mänsson
**The role of osteopontin in tumor progression and metastasis in breast cancer**
Cancer Epidemiol. Biomark. Prev., 16 (2007), pp. 1087-1097
CrossRef View Record in Scopus

Schultz et al., 2009 J. Schultz, P. Lorenz, S.M. Ibrahim, *et al.*
**The functional 443T/C osteopontin promoter polymorphism influences osteopontin gene expression in melanoma cells via binding of c-Myb transcription factor**
Mol. Carcinog., 48 (2009), pp. 14-23
CrossRef View Record in Scopus

Senger et al., 1996 D.R. Senger, S.R. Ledbetter, K.P. Claffey, *et al.*
**Stimulation of endothelial cell migration by vascular permeability factor/vascular endothelial growth factor through cooperative mechanisms involving the alphavbeta3 integrin, osteopontin, and thrombin. Amer**
J. Pathol., 149 (1996), pp. 293-305
View Record in Scopus

Senger et al., 2002 D.R. Senger, C.A. Perruzzi, M. Streit, *et al.*
**The alpha(1)beta(1) and alpha(2)beta(1) integrins provide critical support for vascular endothelial growth factor signaling, endothelial cell migration, and tumor angiogenesis**
Am. J. Pathol., 160 (2002), pp. 195-204
Article Download PDF View Record in Scopus

Shen et al., 2014 Z. Shen, B. Chen, X. Hou, P. Chen, G. Zhao, J. Fan
**Polymorphism- 433 C>T of the osteopontin gene is associated with the susceptibility to develop gliomas and their prognosis in a Chinese cohort**
Cell. Physiol. Biochem., 34 (2014), pp. 1190-1198
CrossRef View Record in Scopus

Shevde and Samant, 2014 L.A. Shevde, R.S. Samant
**Role of osteopontin in the pathophysiology of cancer**

Matrix Biol., 37 (2014), pp. 131-141, 10.1016/j.matbio.2014.03.001
(Epub 2014 Mar 19)
Article Download PDF View Record in Scopus

Sodek et al., 2000 J. Sodek, B. Ganss, M.D. McKee
**Osteopontin**
Crit. Rev. Oral Biol. Med., 11 (2000), pp. 279-303
CrossRef View Record in Scopus

Thalmann et al., 1999 G.N. Thalmann, R.A. Sikes, R.E. Devoll, *et al.*
**Osteopontin: possible role in prostate cancer progression**
Clin. Cancer Res., 5 (1999), pp. 2271-2277
View Record in Scopus

Xiaoxian et al., 2017 Li Xiaoxian, G.M. Oprea-Ilies, U. Krishnamurti
**New developments in breast cancer and their impact on daily practice in pathology**
Arch. Pathol. Lab. Med., 141 (2017), pp. 490-498

Zhang et al., 2015 R. Zhang, W. Yang, Y.C. Li, *et al.*
**The OPN gene polymorphism confers the susceptibility and response to Ara-C based chemotherapy in Chinese AML patients**
Cell. Physiol. Biochem., 35 (2015), pp. 175-183
CrossRef View Record in Scopus

Zhou et al., 2005 Y. Zhou, D.L. Dai, M. Martinka, *et al.*
**Osteopontin expression correlates with melanoma invasion**
J. Invest. Dermatol., 124 (2005), pp. 1044-1052
Article Download PDF CrossRef View Record in Scopus