8

Tumoral Markers in Prostate Cancer

Noemí Cárdenas-Rodríguez and Esaú Floriano-Sánchez
Sección de Posgrado e Investigación, Instituto Politécnico Nacional
Laboratorio de Bioquímica y Biología Molecular, Escuela Médico Militar
Laboratorio de Neuroquímica, Instituto Nacional de Pediatría
México, D.F.

1. Introduction

In Mexico, in 70% of cases, the prostate cancer (PCa) is found in advanced stage. PCa currently occupies second place in frequency of cancer in men, surpassed only by skin cancer, and is the second principal cause of death in men after of lung cancer (Hall et al., 2005).

Reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) are found in a large number of tumors and in high levels they induce cell death, apoptosis, senescence and angiogenesis (Ushio-Fukai & Nakamura, 2008).

One of the major sources of ROS is NADPH oxidase (NOX). The NOX are a family of enzymes that are found in various tissues. The NOX receives an electron from NADPH generating $O_2^{\bullet-}$(Bánfi et al., 2001). Xia et al, Lim et al. and Brar et al. found that some NOX isozymes increase in association with ROS-production and tumor progression in ovarian and human colon cancer and in DU-145 cells of PCa, respectively (Brar et al., 2003; Lim et al., 2005; Xia et al., 2007).

Cells have different antioxidant systems including low molecular weight antioxidant molecules and various antioxidant enzymes. Superoxide dismutase (SOD) catalyses the dismutation of $O_2^{\bullet-}$ into H_2O_2 that can be transformed into H_2O and O_2 by catalase (CAT) (Genkinger et al., 2006). Mn-SOD is the major antioxidant in the mitochondria and is essential to the vitality of mammalian cells. In many types of tumor cells has been found to contain high levels of Mn-SOD, Cu/Zn-SOD or CAT expression compared to their nonmalignant counterpart such as in human tumor cancer cells of esophageal, gastric, ovary, breast, neuroblastoma, osteosarcoma, melanoma, pleura and leukemia (Grigolo et al., 1998; Janssen et al., 2000; Starcevic et al., 2003; Qian et al., 2005, López Laur et al., 2008). However, the role of these enzymes in carcinogenesis remains unclear.

On the other hand, iNOS or NOS-2 is an inducible isoform of nitric oxide synthases (NOS). All isoforms of NOS catalyze the reaction of L-arginine, NADPH and oxygen to nitric oxide (NO•), L-citrulline and NADP. NO• is a lipophilic physiological messenger wich regulate a variety of cellular responses and may exert its cellular action by cGMP-dependent as well as by cGMP-independent pathways (Stamler, 1994). The expression of iNOS has been found to be increased in a variety of human cancers such as colon, stomach, brain and breasts cancers (Alderton et al., 2001; Church & Fulton, 2006) by multiple mechanisms that control their activity (Stamler, 1994; Friebe & Koesling, 2003).

Ciclooxygenase-1 and 2 (COX-1/2) catalyze the initial step in the formation of prostaglandins (Smith & Langenbach, 2001). Very recently their role in carcinogenesis has become more evident. They influence apoptosis, angiogenesis, and invasion, and play a key role in the production of carcinogens. Usually, a high level of COX expression is found in cancer cells (Dannenberg & Zakim, 1999). The role of COX-2 in carcinogenesis has been recently described. Multiple lines of evidence confirm that selective COX-2 inhibitors reduce prostaglandin production and the risk of colorectal, skin and other neoplasias (Sonoshita et al., 2001). COX-2 is related to the formation of carcinogens, tumor promotion and inhibition of apoptosis, angiogenesis and the metastatic process (Ebehart et al.,1994; Uefuji et al., 2000). However, the interactions and links between lipid metabolism and cancer progression remain to be elucidated.

Therefore, in the present study, we decided to evaluate and compare, for the first time, the pattern protein expression of p22 *phox* subunit of NOX, Mn-SOD, Cu/Zn-SOD, CAT, iNOS and COX-2 protein expression in patients with PCa and with BPH.

2. Patients and methods

We obtained 62 samples of prostate tissue through of various surgical procedures (transurethral resection and biopsy transrectal). Approval was obtained from the local research and ethics committee for use of tissue. Of these samples, 30 patients (48.4%) had a diagnosis of PCa, while as 32 patients (51.6%) had a diagnosis of BPH (Department of Medical Urology, Hospital Central Militar, Mexico). The sample collection was conducted from January 2006 to December 2009 and was considered inclusion, exclusion and elimination criteria.

In the PCa group the average age was of 65.3 years and the concentration of preoperative PSA was of 8.6 ng/mL. In this group, the patients were classified according to Gleason scale. The score was of 4 in 1 case (3.3%), 6 in 19 cases (63.3%), 7 in 9 cases (30%) and 8 in 1 case (3.3%). None of the patients had undergone chemotherapy or radiotherapy before surgery.

In the BPH group the average age was of 66.5 years and the concentration of preoperative PSA was of 8.7 ng/mL.

Tissues obtained (500 mg) were stored at -83°C (Revco® Legaci ULT2186 3-35 Dupont SVVA Refrigerants) until further processing.

2.1 Immunohistochemistry

For light microscopy, tissue samples of PCa and BPH were fixed by immersion in formalin (pH 7.4) and embedded in paraffin. Serial cuts of 3 mm of thickness were mounted on poli-L-lisina coated slides (Sigma, St Louis, MO). Sections were initially deparaffinized by washing in xylene and decreasing ethanol concentrations and boiled in Declere (Cell Marque, Hot Springs, AR) to unmask antigen sites. Slides were washed in phosphate buffer saline (PBS). Endogenous peroxidase activity was blocked by exposing slides to 0.6% H_2O_2 in PBS for 30 min.

After of washing in PBS, nonspecific binding was avoided by incubation with 5% blocking solution (5% normal goat serum in PBS) for 20 min. Sections were incubated overnight (16 h) with primary anti-p22 *phox* subunit NOX, anti-Mn-SOD, anti-Cu/Zn-SOD, anti-CAT, anti-iNOS and anti-COX-2 antibody (1:100 for each one). Following removal of the antibodies and repetitive rinsing with PBS, slides were incubated with a biotinylated goat anti-IgG secondary antibodies (1:500 fur each one) (Jackson ImmunoReseach, West Grove, PA). Immunocytochemical identification of positive cells was performed by the use of an avidin-

biotinylated peroxidase complex (ABC-kit Vectastain, Vector Laboratories, Burlingame, CA) and diaminobenzidine (Vector Laboratories, Burlingame, CA). After of intensive washing in PBS, slides were counterstained with hematoxylin. Sections were dehydrated in graded alcohols, treated with xylene and subsequently mounted. All specimens were examined by light microscopy (Axiovert 200 M, Carl Zeiss, Germany), photographs were taken with a digital camera (Axiocam HRC, Carl Zeiss, Germany). The number of positive cells (brown) was determined with a computerized image analyzer KS-300 3.0 (Carl Zeiss, Germany). The percentage of damaged area with histopathological alterations was obtained (400x magnification). Five random fields were studied (total area 1,584,000 μ^2). The results were expressed as a percentage.

2.2 Statistics

Findings were expressed as the mean ± SD. The statistical significance of the protein expression levels of p22 *phox* subunit of NOX, Mn-SOD, Cu/Zn-SOD, CAT, iNOS and COX-2 between PCa and BPH groups glands or stroma, was determined using the software Prism version 3.32 (GraphPad Prism 4.0 Software, San Diego, CA, USA) with "student t-test". It was considered a $p < 0.05$ as statistical difference between groups.

3. Results

The results obtained in PCa and BPH groups are summarized in Table 1. NOX, Mn-SOD, Cu/Zn-SOD and CAT protein immunohistochemistry were significantly higher (1.76, 1.7, 1.78 and 5.88 fold, respectively) in stroma and were significantly higher (3.74, 1.69, 4.76 and 1.59 fold ,respectively) in gland of patients with PCa than that in patients with BPH.
Moreover, NOX, Mn-SOD and CAT protein expressions were significantly higher in gland than in stroma, while as Cu/Zn-SOD protein expression was significantly higher in stroma than in gland in patients with BPH. NOX and Mn-SOD protein expression were significantly higher in gland than in stroma in patients with PCa.
However, iNOS and COX-2 protein expressions were significantly higher in stroma and gland of BPH (1.47 and 2.9 fold, respectively) in comparison with PCa.

Parameters	BPH (n=32)		PCa (n=30)	
	Stroma	Gland	Stroma	Gland
NOX	4.8 ± 1.9	6.7 ± 2[a]	8.45 ± 1.7[c]	25.08 ± 3.5[d,b]
Mn-SOD	11.97 ± 1.6	14.73 ± 1.4[c]	20.45 ± 2.1[c]	24.83 ± 1.7[d,b]
Cu/Zn-SOD	30.3 ± 6.6[d]	11.1 ± 1.9	54.1 ± 14.6[c]	52.8 ± 8.8[d]
CAT	9.8 ± 1.5	37.9 ± 4.5[c]	57.6 ± 15.5[c]	60.1 ± 4.5[d]
iNOS	23.3 ± 8.8[e]	24.6 ± 6.3[f]	15.9 ± 7.1	16.3 ± 4.6
COX-2	12.1 ± 1.3[g]	14.8 ± 2.1[h]	7.4 ± 0.9	5.12± 0.7

[a]P=0.0002 vs stroma BPH; [b]P<0.0001 vs stroma PCa; [c]P<0.0001 vs stroma BPH; [d]P<0.0001 vs gland BPH; [e]P=0.0072 vs stroma PCa; [f]P=0.0016 vs gland PCa; [g]P=0.0314 vs stroma PCa; [h]P=0.0072 vs gland PCa

Table 1. Mean ± SD NOX, Mn-SOD, Cu/Zn-SOD, CAT, iNOS and COX-2 protein expressions (%) in PCa and BPH group.

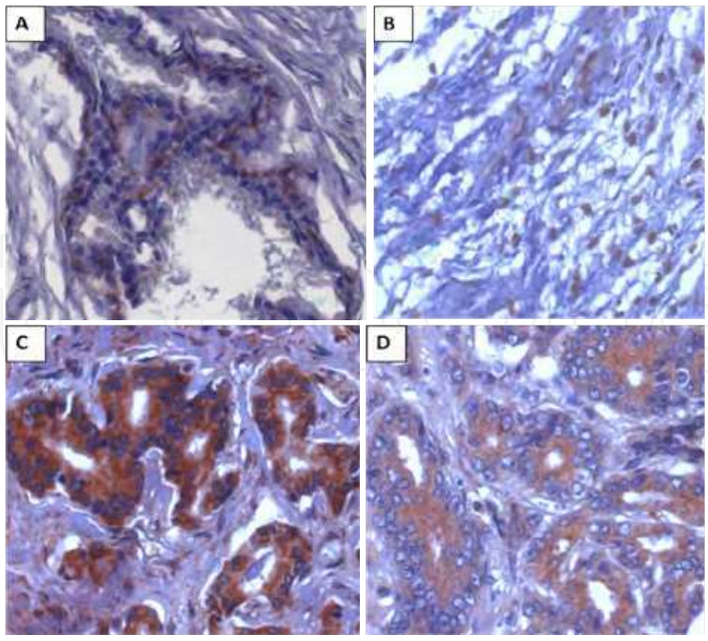


Fig. 1. Immunohistochemical determination of p22 phox subunit of NOX and Mn-SOD in BPH and PCa. (A) y (B) gland of BPH of NOX and Mn-SOD. (C) y (D) gland of PCa of NOX and Mn-SOD. In both groups was determined % area marked by field (400x) and was analized the values with significative increase in gland PCa immunoreactivity.

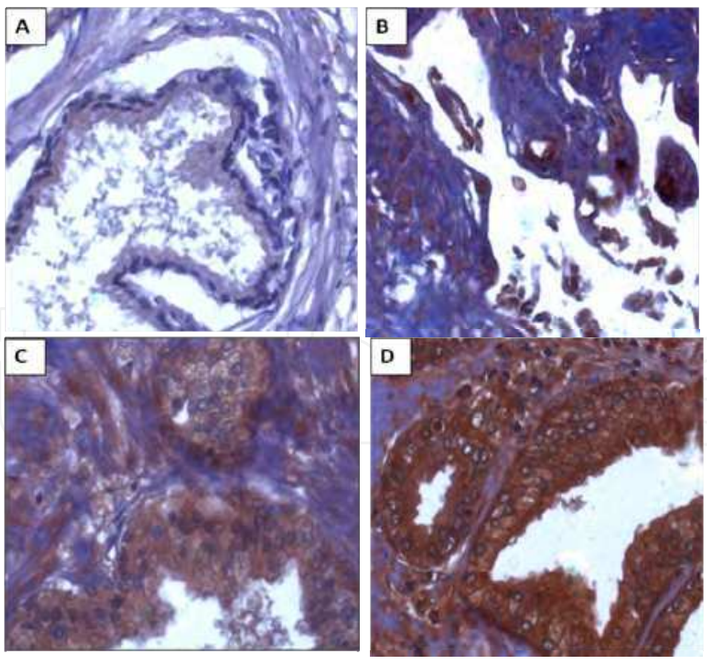


Fig. 2. Immunohistochemical determination of Cu/Zn-SOD and CAT in BPH and PCa. (A) y (B) gland of BPH of Cu/Zn-SOD and CAT. (C) y (D) gland of PCa of Cu/Zn-SOD and CAT. In both groups was determined % area marked by field (400x) and was analized the values with significative increase in gland PCa immunoreactivity.

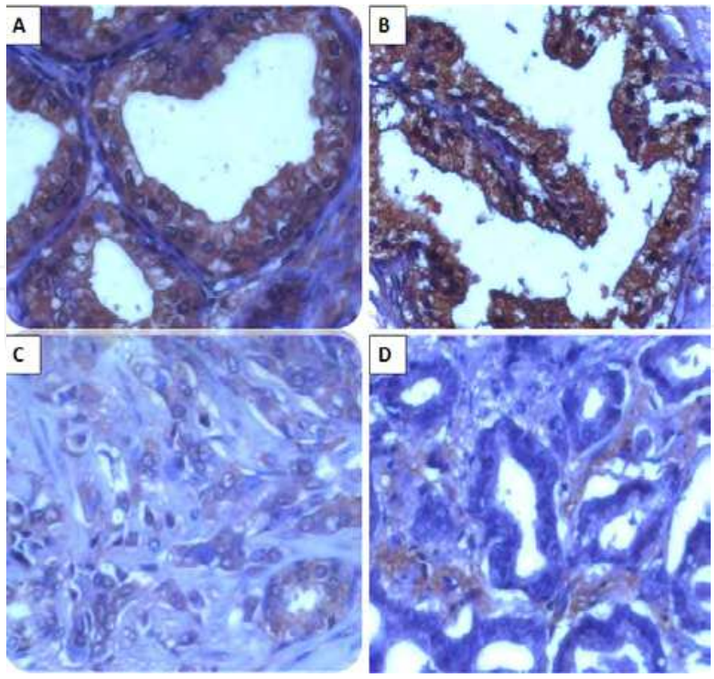


Fig. 3. Immunohistochemical determination of iNOS and COX-2 in BPH and PCa. (A) y (B) gland of BPH of iNOS and COX-2. (C) y (D) gland of PCa iNOS and COX-2. In both groups was determined % area marked by field (400x) and was analized the values with significative increase in gland PCa immunoreactivity.

4. Discussion

Recently, a new hypothesis has been proposed for prostate carcinogenesis. It suggested that exposure to environmental factors such as infectious agents and dietary carcinogens, and hormonal imbalances lead to injury of the prostate and to the development of chronic inflammation and regenerative 'risk factor' lesions, referred to as proliferative inflammatory atrophy (PIA). PCa is associated with oxidative stress, which stimulates the production of reactive oxidative species (ROS) and reactive nitrogen species. Oxidative stress derived from endogenous and exogenous sources are associated with DNA damage that occurs with aging and plays a role in carcinogenesis (Klein et al., 2006).

The results obtained, for the first time, in this study showed an increased in the expression of p22 *phox* subunit of NOX, Mn and Cu/Zn-SOD and CAT in stroma and gland of PCa.

In previous studies concluded that NOX has a role as a signaling mechanism that regulates the cell growth and apoptosis in PCa (Vignais, 2002). The exact signaling pathways of NOX are uncertain and may be tissue specific.

Angiotensin II stimulates the activity of NOX in vascular smooth muscle via protein kinase and NF-κB in the airways and in melanomas (Arnold et al., 2001). Arbiser et al. demonstrated that NOX-1-induced vascular endothelial growth factor (VEGF) and VEGF receptor expression promoting the angiogenesis and rapid expansion of the tumors (Arbiser et al., 2002). Babior BM found that the high levels of ROS are produced spontaneously in PCa and in ovarian cancer. This high production of reactive species was inhibited using an inhibitor of NOX, the diphenyl iodonium (DPI) and the inhibitor of mitochondrial electron

chain, rotenone (Babior, 1999). This suggest that NOX could promote angiogenesis in the early stages of PCa.

By controlling $O_2^{\bullet-}/H_2O_2$ levels, SOD appears to be a critical enzyme in cancer progression. Bravard et al and St Clair et al suggested to the Mn-SOD as a potential tumoral suppressor that might also be involved in cellular differentiation (Zhao et al., 2001).

We suggested that any mutation or epigenetic changes in Mn-SOD gene are the cause of the high level found in the Mn-SOD expression in PCa in the mitochondria. This could have potential effects on survival and proliferation of tumor cells, a fact which has been found in other tumors with aggressive behavior and with a poor prognosis for the patient.

MnSOD polymorphisms have been investigated in several types of malignancies, such as lung, breast and skin cancer (Liu et al., 2004; Han et al., 2007; Bewick et al., 2008). There are at least two functional validated single nucleotide polymorphisms in Mn-SOD. One of these variants is a change in the amino acid codon 9 from valine (GTT) to alanine (GCT) and another is a change in the amino acid codon 16 from valine (GTT) to alanine (GCT) (Tugcu et al., 2007). These changes alter the secondary structure of the protein, affect the transport of the enzyme into mitochondria and reduce the enzymatic activity of Mn-SOD, leaving the cell vulnerable to oxidative damage.

Our results suggest that Mn-SOD probably plays an important role in resistance to treatment of various tumors or in the evolution of invasive tumors.

Brown et al demonstrated an essential role of $O_2^{\bullet-}$ in the posttranslational activation of Cu/Zn-SOD and in the ratio of active to inactive Cu/Zn-SOD, which may be relevant to various diseases, including cancer (Brown et al., 2004). Therefore, $O_2^{\bullet-}$ production by NOX could be induce protein over-expression of Cu/Zn-SOD in PCa.

CAT plays an integral role in the primary defense against oxidative stress by converting H_2O_2 into H_2O and O_2. Genetic polymorphisms of CAT can change expression levels of the protein. A −262C → T polymorphism in the promoter region of the CAT gene is associated with risk of several conditions related to oxidative stress. A transcription factor binding site search indicates that the -262 C allele is located in close proximity to several binding sites for transcription factors and could potentially influence rates of transcription. Forsberg et al. previously showed that the T allele was associated with greater CAT protein levels in some tissues than the C allele (Forsberg et al., 2001). However, different regulatory mechanism of CAT in PCa should be explained.

Our results showed different expressions in NOX, Mn-SOD, Cu/Zn-SOD and CAT in stroma and gland in PCa and BPH groups. The increase of NOX and Mn-SOD expression in gland of PCa and BPH group may have been due to excessive $O_2^{\bullet-}$ and H_2O_2 production that stimulate migration, invasion and angiogenesis of the tumor cells in response to the intracellular changes in ROS levels in this prostate component. The differences in the architecture of the prostate are most likely related to changes in the tumor invasion process

Furthermore our results suggest that exist alterations in the prooxidative-antioxidative balance in PCa, this imbalance is known to alter cellular redox processes, growth, and proliferation and cell cycles, since it is known that certain free radicals mediate the activation of cellular transduction, of transcription factors such as Fos, Jun and nuclear factor kB and an increase in mitochondrial activity in the cells. Moreover, transcription factors such as Rac1, Ref-1 and p53 regulated by ROS are involved in angiogenesis (Ushio-Fukai & Nakayama, 2008).

NO• is synthesized by three differentially gene-encoded NOS in mammals: neuronal NOS (nNOS or NOS-1), inducible NOS (iNOS or NOS-2) and endothelial NOS (eNOS or NOS-3). All three isoforms present similar structures and catalytic modes. The expression of NOS-2 is induced by inflammatory stimuli while NOS-1 and NOS-3 are more or less constitutively expressed. The active form of NOS-1 and -3 requires two NOS monomers associated with two Ca^{2+}-binding protein calmodulin and cofactors such as (6R)-5,6,7,8-tetrahydrobiopterin (BH_4), FAD, FMN and haem group and catalyze the reaction of L-arginine, NADPH and oxygen to NO•, L-citrulline and NADP (Alderton et al., 2001; Stuehr et al., 2004). NOS isoforms are differentially regulated at transcriptional, translational and post-translational levels. The intracellular localization is relevant for NOS activity. Evidence indicates that NOS are present in plasma membrane, Golgi, cytosol, nucleus and mitochondria (Oess et al., 2006; Iwakiri et al., 2006). The expression of iNOS can be transcriptionally regulated by factors such as cytokines (e.g. interferon-γ (IFN- γ), interleukin-1β (IL-1 β) and tumour necrosis factor-α (TNF-α), bacterial endotoxin (LPS) and oxidative stress (e.g. under conditions encountered during hypoxia)(Xu & Liu, 1998).

An initial study on iNOS expression in human breast cancer suggested that iNOS activity was higher in less differentiated tumours in a panel of 15 invasive breast carcinomas (Thomsen et al., 1995). Reveneau et al reported NOS activity in 27 of 40 tumours studied (Reveneau et al., 1999). Vakkala et al showed that carcinomas with both iNOS positive tumour and stromal cells had a higher apoptotic index and a higher calculated microvessel density index (Vakkala et al., 2000). Loibl et al further demonstrated that while none of the benign lesions were positive for iNOS, 67% *in situ* carcinomas and 61% invasive lesions showed iNOS tumour cell staining (Loibl et al., 2002).

In addition to breast cancer, iNOS has also been shown to be markedly expressed in approximately 60% of human adenomas and in 20-25% of colon carcinomas, while expression was either low or absent in the surrounding normal tissues (Ambs et al., 1998a). In human ovarian cancer, iNOS activity has been localized in tumour cells and not found in normal tissue (Thomsen et al., 1995). Other tumours that have demonstrated iNOS gene expression are brain, head and neck, esophagus, lung, prostate, bladder, pancreatic, and Kaposi's sarcoma (Cobbs et al., 1995; Rosbe et al., 1995; Wilson et al., 1998; Ambs et al., 1998a; Klotz et al., 1998; Hajri et al., 1998; Weninger et al., 1998; Swane et al., 1999).

In this study, we found that exist strong expression of iNOS in stroma and gland of HPB, in comparison with PCa. It Have been demonstrated that NO•- mediated up-regulation of VEGF. In the results is possible that NO• generated by iNOS in stroma may promote early new blood vessel formation by up-regulating VEGF and enhance ability of the tumour to grow and increases its invasiveness ability in gland. (Ambs et al., 1998a). Moreover, the accumulation of p53 in gland can result in down-regulation of iNOS expression by inhibition of iNOS promoter activity (Ambs et al., 1998b). On the other hand, the generation of chronic injury and irritation initiate the inflammatory response of stroma to gland (NOS-1). A subsequent respiratory burst an increase uptake of oxygen that leads to the release of reactive oxygen species (NO•, $ONOO^-$, N_2O_3, NO_2 and NO_3) from leucocytes can damage surrounding cells and drive carcinogenesis by altering targets and pathways that are crucial to normal prostate homeostasis (Coussens & Werb, 2002; Fukumura et al., 2006).

COX-1 and COX-2 regulate a key step in prostanoid (i.e., tromboxanes and prostaglandins) synthesis. Prostaglandins regulate various pathophysiological processes such as inflammatory reaction, gastrointestinal cytoprotection and ulceration (Smith & Langenbach,

2001). COX-1 is the constitutive isoform and COX-2 is the inducible isoform. COX-1 is expressed in most tissues and plays a role in the production of prostaglandins that control normal physiological processes. COX-2 is undetectable in most normal tissues (except for the central nervous system, kidneys and seminal vesicles), but is induced by various inflammatory and mitogenic stimuli (growth factors, pro-inflammatory cytokines and tumor necrosis factor) and other regulatory factors (Peppelenbosch et al., 1993; Zhang et al., 1998, Chen et al., 2001; Dempke et al., 2001). Although the mechanism of COX-2 upregulation is not fully understood, it could result from activation of Ras and mitogen-activated protein kinase (MAPK) pathway. It has been recognized that Akt/PKB activity is implicated in Ras-induced expression of COX-2. COX-2 is regulated at transcriptional and post-transcriptional levels by proinflamatory agents. These pathways lead to the activation of regulatory factors that eventually bind the promoter region of the COX-2 gene. (Sheng et al., 1998, 2000).

In this study, we found that exist strong expression of COX-2 in stroma and gland of HPB, in comparison with PCa. There are conflicting data regarding whether COX-2 is increased in the epithelial , gland or the stromal component of tumors (Horsman et al., 2010). Liu et al were the first to describe tumorigenesis induced by COX-2 over-expression. In their study, the murine COX-2 gene was inserted downstream of a murine mammary tumor virus promoter. As a consequence, hyperplasia and carcinoma of the mammary gland were observed and associated with strong COX-2 expression in mammary gland epithelial cells with increase prostaglandin E2 levels. (Liu et al., 2001). The role of COX-2 in tumor promotion is more strongly supported by previous studies in colorectal tumor models describen by Oshima et al (Oshima et al., 1996). These findings have been confirmed analyzing many tumors including pancreas, skin, gastric, bladder, lung, head, and neck cancers, suggesting that COX-2, but not COX-1, may play a pivotal role in tumor formation and growth (Thun et al., 2002). COX-2-derived prostaglandins contribute to tumor growth by inducing angiogenesis that sustain tumor cell viability and growth. COX-2 is expressed within human tumor neovasculature as well as in neoplastic cells present in human colon, breast, prostate and lung cancer biopsy tissue. (Kerbel & Folkman, 2002). The proangiogenic effects of COX-2 are mediated primarily by three products of arachidonic metabolism: Tromboxane A2, Prostaglandins I2 and E2 and selective inhibition of COX-2 activity has been shown to suppress angiogenesis *in vitro* and *in vivo* (Tsujii et al., 1998; Masferrer et al., 2000; Uefuji et al., 2000). We suggested that COX-2 overexpression in stroma inhibit apoptosis and promote angiogenesis in prostate gland.

Our results suggest that iNOS and COX-2 play a key role in tumorigenesis and indicate that iNOS and COX-2-selective inhibitors could be a novel class of therapeutic agents for PCa.

5. Conclusions

We suggested that the $O_2^{\bullet-}/H_2O_2$ balance regulated by the over-expression of NOX, Cu/Zn-SOD, Mn-SOD and CAT is actively involved in tumor environment, cell proliferation, differentiation, tumor progression and angiogenesis of PCa. On the other hand, iNOS and COX-2 may promote blood vessel formation in gland from its over-expression in stroma by multiple mechanisms that involve reactive oxygen species, transcription factors, cytokines, growth factors and tumor necrosis factor.

Moreover, we suggested that the NOX, Cu/Zn-SOD, Mn-SOD, CAT, iNOS and/or COX-2 in combination with PSA, could be a molecular markers or prognostic indicators for the early diagnosis and post-treatment monitoring of PCa.

6. Future research

Our research group is determining the gene expression and activity of nitric oxide synthases isoforms (eNOS, nNOS and iNOS), Mn-SOD, Cu/Zn-SOD, Glutathione peroxidase, Glutathione reductase, Glutathione-S-transferase, Catalase and Ciclooxygenase-2 to integrate the effect of the regulation of the antioxidant system in the development of prostate cancer and recently in breast cancer.

Actually, we begin a new line of research where we studied the gene expression and polymorphisms of some components of the cytochrome P450 system as well as its association with the risk of developing prostate cancer and breast cancer. We found a protein over-expression of CYP2W1, 4F11 and 8A1, orphans cytochromes, in prostate cancer. We hope to find a molecular marker or prognostic indicator for prostate cancer and breast cancer.

7. References

[1] Alderton, W.K.; Cooper, C.E. & Knowles, R.G. (2001). Nitric oxide synthases: structure, function and inhibition. *Journal of Biochremistry,* Vol. 357, No. 3, (August 2001), pp. 593-615, ISSN 0021-924X

[2] Ambs, S.; Merriam, W.G.; Bennett, W.P.; Felley-Bosco, E.; Ogunfusika, M.O.; Oser, S.M.; Klein, S.; Shields, P.G.; Billiar, T.R. & Harris, C.C. (1998a) Frequent nitric oxide synthase-2 expression in human colon adenomas: implication for tumor angiogenesis and colon cancer progression. *Cancer Research*, Vol. 58, No. 2, (January 1998), pp. 334-341, ISSN 1538-7445

[3] Ambs, S.; Ogunfusika, M.O.; Merriam, W.G.; Bennett, W.P.; Billiar, T.R. & Harris, C.C. (1998b). Up-regulation of inducible nitric oxide synthase expression in cancer-prone p53 knockout mice. *Proceedings of the National Academy of Sciences U S A,* Vol. 95, No. 15, (July 1998), pp. 8823-8828, ISSN 0027-8424

[4] Arbiser, J.L.; Petros, J; Klafter, R.; Govindajaran, B.; McLaughlin, E.R.; Brown, L.F.; Cohen, C.; Moses, M.; Kilroy, S.; Arnold, R.S. & Lambeth, J.D. (2002). Reactive oxygen generated by Nox1 triggers the angiogenic switch. *Proceedings of the National Academy of Sciences U S A,* Vol. 99, No 2, (January 2002), pp. 715-720, ISSN 0027-8424

[5] Arnold, R.S.; Shi, J.; Murad, E.; Whalen, A.M.; Sun, C.Q.; Polavarapu, R.; Parthasarathy, S.; Petros, .JA. & Lambeth, J.D. (2001). Hydrogen peroxide mediates the cell growth and transformation caused by the mitogenic oxidase Nox1. *Proceedings of the National Academy of Sciences U S A,* Vol. 98, No 10, (May 2001), pp. 5550-5555, ISSN 0027-8424

[6] Babior, B.M. (1999). NADPH oxidase: an update. *Blood,* Vol. 92, No. 5, (March 1999), pp. 1454–1476, ISSN 0006-4971

[7] Bánfi, B.; Molnár, G.; Maturana, A.; Steger, K.; Hegedûs, B.; Demaurex, N. & Krause, K.H. (2001). A Ca^{2+}-activated NADPH oxidase in testis, spleen, and lymph nodes. The *Journal of Biological Chemistry,* Vol. 276, No. 40, (October 2001), pp. 37594-37601, ISSN 0021-9258

[8] Bewick, M.A.; Conlon, M.S. & Lafrenie, R.M. (2008). Polymorphisms in manganese superoxide dismutase, myeloperoxidase and glutathione-*S*-transferase and survival

after treatment for metastatic breast cancer. *Breast Cancer Research and Treatment,* Vol. 111, No. 1, (September 2008), pp. 93-101, ISSN 0167-6806

[9] Brar, S.S.; Corbin, Z.; Kennedy, T.P.; Hemendinger, R.; Thornton, L.; Bommarius, B.; Arnold, R.S.; Whorton, A.R.; Sturrock, A.B.; Huecksteadt, T.P.; Quinn, M.T.; Krenitsky, K.; Ardie, K.G.; Lambeth, J.D. & Hoidal, J.R. (2003). NOX5 NAD(P)H oxidase regulates growth and apoptosis in DU 145 prostate cancer cells. *American Journal of Physiology.Cell Physiology,* Vol. 285, No. 2, (August 2003), pp. C353-C369, ISSN 0363-6143

[10] Brown, N.M.; Torres, A.S.; Doan Pe, & O`Halloran, T.V. (2004). Oxygen and the copper chaperone CCS regulate posttranslational activation of Cu, Zn superoxide dismutase. *Proceedings of the National Academy of Sciences U S A,* Vol. 101, No. 15, (April 2004), pp. 5518–5523, ISSN 0027-8424

[11] Chen, C.C.; Sun, Y.T.; Chen, J.J. & Chang, Y.J. (2001). Tumor necrosis factor-induced cyclooxygenase-2 expression via sequential activation of ceramide-dependent mitogenactivated protein kinases, and I_B kinase 1/2 in human alveolar epithelial cells. *Molecular Pharmacology* Vol. 59, No. 3, (March 2001), pp. 493–500, ISSN 0026-895X

[12] Church, J.E. & Fulton, D. (2006). Differences in eNOS activity because of subcellular localization are dictated by phosphorylation state rather than the local calcium environment. *The Journal of Biological Chemistry,* Vol. 281, No. 3, (October 2005), pp. 1477-1488, ISSN 0021-9258

[13] Cobbs, C.S.; Brenman, J.E.; Aldape, K.D.; Bredt, D.S. & Israel, MA. (1995). Expression of nitric oxide synthase in human central nervous system tumors. *Cancer Research*, Vol. 55, No. 4, (February 1995), pp. 727-730, ISSN 1538-7445

[14] Coussens, L.M. & Werb, Z. (2002). Inflammation and cancer. *Nature,* Vol. 420, No. 6917, (December 2002), pp. 860-867, ISSN 0028-0836

[15] Dannenberg, A.J. & Zakim, D. (1999). Chemoprevention of colorectal cancer through inhibition of cyclooxygenase-2. *Seminars in Oncology*, Vol. 26, No. 5, (October 1999), pp. 499–504, ISSN 0093-7754

[16] Dempke, W.; Rie, C.; Grothey, A. & Schmoll, H.J. (2001). Cyclooxygenase-2: a novel target for cancer chemotherapy? *The Journal of Cancer Research of Clinical On*cology, Vol. 127, No. 7, (July 2001), pp. 411–417, ISSN 0171-5216

[17] Eberhart, C.E.; Coffey, R.J.; Radhika, A.; Giardiello, F.M.; Ferrenbach, S. & DuBois, R.N. (1994) Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology*, Vol. 107, No. 4, (October 1994), pp. 1183–1188, ISSN 0016-5085

[18] Forsberg, L.; Lyrenas, L.; de Faire, U. & Morgenstern, R. (2001). A common functional C-T substitution polymorphism in the promoter region of the human catalase gene influences transcription factor binding, reporter gene transcription and is correlated to blood catalase levels. *Free Radical Biology & Medicine,* Vol. 30, No. 5, (March 2001), pp. 500 –505, ISSN 0891-5849

[19] Friebe, A. & Koesling, D. (2003). Regulation of nitric oxide-sensitive guanylyl cyclase. *Circulation Research,* Vol. 93, No. 2, (July 2003), pp. 96-105, ISSN 00097330

[20] Fukumura, D.; Kashiwagi, S. & Jain, R.K. (2006). The role of nitric oxide in tumour progression. *Nature Reviews Cancer*, Vol. 6, No. 7, (July, 2006), pp. 521-534, ISSN 1474-175X

[21] Genkinger, J.M.; Platz, E.A.; Hoffman, S.C.; Strickland, P.; Huang, H.Y.; Comstock, G.W. & Helzlsouer, K.J. (2006). C47T polymorphism in manganese superoxide dismutase (MnSOD) antioxidant intake and survival. *Mechanisms of Ageing and Development,* Vol. 127, No. 4, (April 2006), pp. 371-377, ISSN 0047-6374

[22] Grigolo, B.; Lisignoli, G.; Toneguzzi, S.; Mazzetti, I. & Facchini, A. (1998). Copper/zinc superoxide dismutase expression by different human osteosarcoma cell lines. Anticancer Research, Vol. 18, No. 2A, (March-April 2008), pp. 1175-1180, ISSN 0250-7005

[23] Hajri, A.; Metzger, E.; Vallat, F.; Coffy, S.; Flatter, E.; Evrard, S.; Marescaux, J. & Aprahamian, M. (1998). Role of nitric oxide in pancreatic tumour growth: in vivo and in vitro studies. *British Journal of Cancer,* Vol. 78, No. 7, (October 1998), pp. 841-849, ISSN 0007-0920.

[24] Hall, S.E.; Holman, C.D.; Wisniewsk, Z.S. & Semmens, J. (2004).Prostate cancer: socio-economic, geographical and private-health insurance effects on care and survival *British Journal of Urology,* Vol.95, No. 1, (January 2005), pp. 51-58, ISSN 0007-1331

[25] Han, J.; Colditz, G.A. & Hunter, D.J. (2007). Manganese superoxide dismutase polymorphism and risk of skin cancer (United States). *Cancer Causes Control,* Vol. 18, No. 1, (February 2007), pp. 79-89, ISSN 0957-5243

[26] Horsman, M.R.; Bohn, A.B. & Busk, M. (2010). Vascular targeting therapy: potential benefit depends on tumor and host related effects. *Experimental Oncology*, Vol. 32, No. 3, (September 2010), pp. 143-148, ISSN 1812-9269

[27] Iwakiri, Y.; Satoh, A.; Chatterjee, S.; Toomre, D.K.; Chalouni, C.M.; Fulton, D.; Groszmann, R.J.; Shah, V.H. & Sessa, W.C. (2006). Nitric oxide synthase generates nitric oxide locally to regulate compartmentalized protein S-nitrosylation and protein trafficking. *Proceedings of the National Academy of Sciences U S A*, Vol. 103, No. 52, (December 2006), pp. 19777-19782, ISSN 0027-8424

[28] Janssen, A.M.; Bosman, C.B.; van Duijn, W.; Oostendorp-van de Ruit, M.M.; Kubben, F.J.; Griffioen, G.; Lamers, C.B.; van Krieken, J.H.; van de Velde, C.J. & Verspaget, H.W. (2000). Superoxide dismutases in gastric and esophageal cancer and the prognostic impact in gastric cancer. *Clinical Cancer Research*, Vol. 6, No. 8, (August 2000), pp. 3183-3192, ISSN 1078-0432

[29] Kerbel, R. & Folkman, J. (2002). Clinical translation of angiogenesis inhibitors. *Nature Reviews Cancer,* Vol. 2, No. 10, (October 2002), pp. 727–739, ISSN 1474-175X

[30] Klein, E.A.; Casey, G. & Silverman, R. (2006). Genetic susceptibility and oxidative tress in prostate cancer: integrated model with implications for prevention. *Urology,* Vol. 68, No 6, (December 2006), .pp. 1145–1151, ISSN 0090-4295

[31] Klotz, T.; Bloch, W.; Volberg, C.; Engelmann, U. & Addicks, K. (1998). Selective expression of inducible nitric oxide synthase in human prostate carcinoma. *Cancer,* Vol. 82, No. 10, (May 1998), pp. 1897-903, ISSN 1097-0142

[32] Lim, S.D.; Sun, C.; Lambeth, J.D.; Marshall, F.; Amin, M.; Chung, L.; Petros, J.A. & Arnold, R.S. (2005) Increased Nox1 and hydrogen peroxide in prostate cancer. *Prostate,* Vol. 62, No. 2, (February 2005), pp. 200–207, ISSN 0270-4137

[33] Liu, C.H.; Chang, S.H.; Narko, K.; Trifan, O.C.; Wu, M.T.; Smith, E.; Haudenschild, C.; Lane, T.F. & Hla, T. (2001). Over-expression of cyclooxygenase-2 is sufficient to induce tumorigenesis in transgenic mice. The *Journal of Biological Chemistry*, Vol. 276, No. 1, (January 2001), pp. 18563–18569, ISSN 0021-9258

[34] Liu, G.; Zhou, W.; Park, S.; Wang, L.I.; Miller, D.P.; Wain, J.C.; Lynch, T.J.; Su, L. & Christiani, D.C. (2004). The SOD2 Val/Val genotype enhances the risk of non small cell lung carcinoma by p53 and XRCC1 polymorphisms. *Cancer,* Vol. 101, No. 12, (December 2004), pp. 2802-2808, ISSN 0008-543X

[35] Loibl, S.; von Minckwitz, G.; Weber, S.; Sinn, H.P.; Schini-Kerth, V.B; Lobysheva, I.; Nepveu, F.; Wolf, G.; Strebhardt, K. & Kaufmann, M. (2002). Expression of endothelial and inducible nitric oxide synthase in benign and malignant lesions of the breast and measurement of nitric oxide using electron paramagnetic resonance spectroscopy. *Cancer* , Vol. 95, No. 6, (September 2002), pp. 1191-1198, ISSN 1097-0142

[36] López Laur, J.D.; Abud, M.; López Fontana, C.; Silva, J.; Cisella, Y.; Pérez Elizalde, R. & Ortiz, A. (2008). Antioxidant power and cellular damage in prostate cancer. *Archivos Españoles de Urología*, Vol. 61, No. 5, (June 2008), pp. 563-569, ISSN 0004-0614

[37] Masferrer, J.L.; Leahy, K.M.; Koki, A.T.; Zweifel, B.S.; Settle, S.L.; Woerner, B.M.; Edwards, D.A.; Flickinger, A.G.; Moore, R.J. & Seibert, K. (2000). Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. *Cancer Research*, Vol. 60, No. 5, (March 2000), pp. 1306–1311, ISSN 1538-7445

[38] Oess, S.; Icking, A.; Fulton, D.; Govers, R. & Müller-Esterl, W. (2006). Subcellular targeting and trafficking of nitric oxide synthases. *Journal of Biochremistry*, Vol. 396, No. 3, (June 2006) pp. 401-409, ISSN 0021-924X

[39] Oshima, M.; Dinchuk , J.E.; Kargman, S.L.; Oshima, H.; Hancock, B.; Kwong, E.; Trzaskos, J.M.; Evans, J.F. & Taketo, M.M. (1996). Suppression of intestinal polyposis in *Apc*-knockout mice by inhibition of cyclooxygenase-2 (COX-2). *Cell,* Vol. 87, No. 5, (November 1996), pp. 803–809, ISSN 0092-8674

[40] Peppelenbosch, M.P.; Tertoolen, L.G.; Hage, W.J. & de Laat, S.W. (1993). Epidermal growth factor-induced actin remodeling is regulated by 5-lipoxygenase and cyclooxygenase products. *Cell*, Vol. 74, No. 3, (August, 1993), pp. 565–575, ISSN 0092-8674

[41] Qian, Y.; Zheng, Y.; Abraham, L.; Ramos, K.S. & Tiffany-Castiglioni, E. (2005). Differential profiles of copper-induced ROS generation in human neuroblastoma and astrocytoma cells. *Brain Research. Molecular Brain Research*, Vol. 134, No 2, (April 2005), pp. 323-332, ISSN 0169-328X

[42] Reveneau, S.; Arnould, L.; Jolimoy, G.; Hilpert, S.; Lejeune, P.; Saint-Giorgio, V.; Belichard, C. & Jeannin, J.F. (1999). Nitric oxide synthase in human breast cancer is associated with tumor grade, proliferation rate, and expression of progesterone receptors. Laboratory Investigation, Vol. 79, No. 10, (October 1999), pp. 1215-25, ISSN 0023-6837

[43] Rosbe, K.W.; Prazma, J.; Petrusz, P.; Mims, W.; Ball, S.S. & Weissler, M.C. (1995). Immunohistochemical characterization of nitric oxide synthase activity in squamous cell carcinoma of the head and neck. *Otolaryngology: Head and Neck Surgery*, Vol.113, No. 5, (November 1995), pp. 541-549, ISSN 01945998

[44] Sheng, H.; Shao, J. & Dubois, R.N. (2001). K-Ras-mediated increase in cyclooxygenase 2 mRNA stability involves activation of the protein kinase B. *Cancer Research*, Vol. 61, No. 6, (March 2001), pp. 2670–2675, ISSN 1538-7445

[45] Sheng, H.; Shao, J.; Dixon, D.A.; Williams, C.S.; Prescott, S.M.; DuBois, R.N. & Beauchamp, R.D. (2000). Transforming growth factor-1 enhances Ha-ras-induced expression of cyclooxygenase-2 in intestinal epithelial cells via stabilization of

mRNA. The *Journal of Biological Chemistry*, Vol. 275, No. 9, (March 2000), pp. 6628–6635, ISSN 0021-9258

[46] Smith, W.L. & Langenbach, R. (2001). Why there are two cyclooxygenase isozymes. *The Journal of Clinical Investigation*, Vol. 107, No. 12, (June 2001), pp. 1491–1495, ISSN 0021-9738

[47] Sonoshita, M.; Takaku, K.; Sasaki, N.; Sugimoto, Y.; Ushikubi, F.; Narumiya, S. & Oshima, M. (2001). Acceleration of intestinal polyposis through prostaglandin receptor EP2 in Apc-knockout mice. *Nature Medicine,* Vol. 7, No. 9, (September 2001), pp. 1048–1051, ISSN 1078-8956

[48] Stamler, J.S. (1994). Redox signaling: nitrosylation and related target interactions of nitric oxide. *Cell,* Vol. 78, No. 3, (September 1994), pp. 931-936, ISSN 0092-8674

[49] Starcevic, S.L.; Diotte, N.M.; Zukowski, K.L.; Cameron, M.J. & Novak, R.F. (2003). Oxidative DNA damage and repair in a cell lineage model of human proliferative breast disease (PBD). *Toxicological Sciences (U.S.),* Vol. 75, No.1, (September 2003), pp. 74-81, ISSN 1096-6080

[50] Stuehr, D.J.; Santolini, J.; Wang, Z.Q.; Wei, C.C. & Adak, S. (2004). Update on mechanism and catalytic regulation in the NO synthases. *The Journal of Biological Chemistry*, Vol. 279, No. 35, (May 2004), pp. 27257-27562, ISSN 0021-9258

[51] Swana, H.S.; Smith, S.D.; Perrotta, P.L.; Saito, N.; Wheeler, M.A. & Weiss, R.M. (1999). Inducible nitric oxide synthase with transitional cell carcinoma of the bladder. *Journal of Urology*, Vol. 161, No. 2, (February 1999), pp. 630-634, ISSN 0022-5347

[52] Thomsen, L.L.; Miles, D.W.; Happerfield, L.; Bobrow, L.G.; Knowles, R.G. & Moncada, S. (1995). Nitric oxide synthase activity in human breast cancer. *British Journal of Cancer*, Vol. 72, No. 1, (July 1995), pp. 41-44, ISSN 0007-0920

[53] Thun, M.J.; Henley, S.J. & Patrono, C. (2002). Nonsteroidal anti-inflammatory drugs as anticancer agents: mechanistic, pharmacologic, and clinical issues," *Journal of the National Cancer Institute*, Vol. 94, No. 4, (February 2002), pp. 252–266, ISSN 0027-8874

[54] Tsujii, M.; Kawano, S.; Tsuji, S.; Sawaoka, H; Hori, M. & DuBois, R.N. (1998). Cyclooxygenase regulates angiogenesis induced by colon cancer cells. *Cell,* Vol. 93, No. 5, (May 1998), pp. 705–716, ISSN 0092-8674

[55] Uefuji, K.; Ichikura, T. & Mochizuki, H. (2000). Cyclooxygenase-2 expression is related to prostaglandin biosynthesis and angiogenesis in human gastric cancer. *Clinical Cancer Research*, Vol. 6, No. 1, (January 2000), pp. 135–138, ISSN 1557-3265

[56] Tugcu, V.; Ozbek, E.; Aras, B.; Arisan, S.; Caskurlu, T. & Tasci, A.I. (2007). Manganese superoxide dismutase (Mn-SOD) gene polymorphisms in urolithiasis. *Urological Research,* Vol. 35, No 5, (October 2007), pp. 219-224, ISSN 0300-5623

[57] Uefuji, K.; Ichikura, T. & Mochizuki, H. Cyclooxygenase-2 expression is related to prostaglandin biosynthesis and angiogenesis in human gastric cancer. *Clinical Cancer Research*, Vol. 6, No. 1, (January 2000), pp. 135–138, ISSN 1557-3265

[58] Ushio-Fukai, M. & Nakamura, Y. (2008). Reactive oxygen species and angiogenesis: NADPH oxidase as target for cancer therapy. *Cancer Letters*, Vol. 266, No. 1, (July 2008), pp. 37-52, ISSN 0304-3835

[59] Vakkala, M.; Kahlos, K.; Lakari, E.; Paakko, P.; Kinnula, V. & Soini, Y. (2000). Inducible nitric oxide synthase expression, apoptosis, and angiogenesis in in situ and invasive breast carcinomas. *Clinical Cancer Research*, Vol. 6, No. 6, (June 2000), pp. 2408-2416, ISSN 1557-3265

[60] Vignais, P.V. (2002). The superoxide-generating NADPH oxidase: structural aspects and activation mechanism. *Cell and Molecular Life Sciences,* Vol. 59, No. 9, (September 2002), pp. 1428–1459, ISSN 1420-682X

[61] Weninger, W.; Rendl, M.; Pammer, J.; Mildner, M.; Tschugguel, W.; Schneeberger, C.; Stürzl, M. & Tschachler, E. (1998). Nitric oxide synthases in Kaposi sarcoma are expressed predominantly by vessels and tissue macrophages. *Laboratory Investigation*, Vol. 78, No. 8, (August 1998), pp. 949-955, ISSN 0023-6837

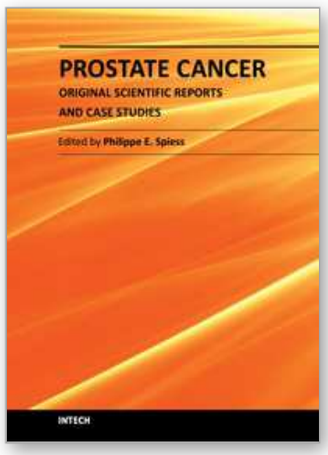
[62] Wilson, K.T.; Fu, S.; Ramanujam, K.S. & Meltzer, S.J. (1998). Increased expression of inducible nitric oxide synthase and cyclooxygenase-2 in Barrett s esophagus and associated adenocarcinomas. *Cancer Research*, Vol. 58, No. 14, (July 1998), pp.:2929-2934, ISSN 1538-7445

[63] Xia, C.; Meng, Q.; Liu, L.Z.; Rojanasakul, Y.; Wang, X.R. & Jiang, B.H. (2007). Reactive oxygen species regulate angiogenesis and tumor growth through vascular endothelial growth factor. *Cancer Research*, Vol. 67, No. 22, (November 2007), pp. 10823-10830, ISSN 0008-5472

[64] Xu, W. & Liu, L. (1998). Nitric Oxide: from a mysterious labile factor to the molecule of the Nobel Prize. Recent progress in nitric oxide research. *Cell Research*, Vol. 8, No. 4, (December 1998), pp. 251-258, ISSN 1001-0602

[65] Zhang, F.; Subbaramaiah, K.; Altorki, N. & Dannenberg, A.J. (1998). Dihydroxy bile acids activate the transcription of cyclooxygenase-2. The *Journal of Biological Chemistry*, Vol. 273, No. 4, (January 1998), pp. 2424-2428, ISSN 0021-9258

[66] Zhao, Y.; Kiningham, K.K.; Lin, S.M. & St Clair, D.K. (2001). Overexpression of MnSOD protects murine fibrosarcoma cells (FSa-II) from apoptosis and promotes a differentiation program upon treatment with 5-azacytidine: involvement of MAPK and NFkappaB pathways. *Antioxidants & Redox Signaling,* Vol. 3, No. 3, (June 2001), pp. 375–386, ISSN 1523-0864



Prostate Cancer - Original Scientific Reports and Case Studies
Edited by Dr. Philippe E. Spiess

ISBN 978-953-307-342-2
Hard cover, 238 pages
Publisher InTech
Published online 21, November, 2011
Published in print edition November, 2011

This book encompasses three sections pertaining to the topics of cancer biology, diagnostic markers, and therapeutic novelties. It represents an essential resource for healthcare professionals and scientist dedicated to the field of prostate cancer research. This book is a celebration of the significant advances made within this field over the past decade, with the hopes that this is the stepping stone for the eradication of this potentially debilitating and/or fatal malignancy.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Noemî Cárdenas-Rodrîguez and Esaú Floriano-Sánchez (2011). Tumoral Markers in Prostate Cancer, Prostate Cancer - Original Scientific Reports and Case Studies, Dr. Philippe E. Spiess (Ed.), ISBN: 978-953-307-342-2, InTech, Available from: http://www.intechopen.com/books/prostate-cancer-original-scientific-reports-and-case-studies/tumoral-markers-in-prostate-cancer

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen