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Expression of Spermidine/Spermine N[1]-Acetyl Transferase (SSAT) in Human Prostate Tissues is Related to Prostate Cancer Progression and Metastasis

Wei Huang[1], **Jens C Eickhoff**[2], **Farideh Mehraein Ghomi**[3], **Dawn R. Church**[3], **George Wilding**[3], and **Hirak S. Basu**[3,*]

[1]Department of Pathology and Laboratory Medicine, University of Wisconsin Carbone Cancer Center, University of Wisconsin-Madison, Madison WI

[2]Department of Biostatistics, University of Wisconsin Carbone Cancer Center, University of Wisconsin-Madison, Madison WI

[3]Department of Medicine, University of Wisconsin Carbone Cancer Center University of Wisconsin, Madison, WI

Abstract

Introduction—PCa in many patients remains indolent for the rest of their lives, but in some patients it progresses to lethal metastatic disease. Gleason Score (GS) is the current clinical method for PCa prognosis. It cannot reliably identify aggressive PCa, when GS is ≤7. It is shown that oxidative stress plays a key role in PCa progression. We have shown that in cultured human PCa cells, an activation of Spermidine/Spermine N[1]-acetyl transferase (EC 2.3.1.57) enzyme initiates a polyamine oxidation pathway and generates copious amounts of reactive oxygen species (ROS) in polyamine-rich PCa cells.

Method—We used RNA *in situ* hybridization (RNA-ISH) and immunohistochemistry (IHC) methods to detect SSAT mRNA and protein expression in two tissue microarrays (TMA) created from patient prostate tissues. We analyzed 423 patient prostate tissues in the two TMAs.

Results—Our data show that there is a significant increase in both SSAT mRNA and the enzyme protein in the PCa cells as compared to their benign counterpart. This increase is even more pronounced in metastatic PCa tissues as compared to the PCa localized in the prostate. In the prostatectomy tissues from early-stage patients, the SSAT protein level is also high in the tissues obtained from the patients who ultimately progress to advanced metastatic disease.

Discussion—Based on these results combined with published data from our and other laboratories we propose an activation of an autocrine feed-forward loop of PCa cell proliferation in the absence of androgen as a possible mechanism of castrate-resistant prostate cancer (CRPCa) growth.

*To whom all communications should be directed at: Room #7068, Wisconsin Institute for Medical Research, 1111 Highland Avenue, Madison, WI, 53705, hsb@medicine.wisc.edu.

Introduction

Prostate cancer (PCa) is the second leading cause of cancer related mortality in US men. Over 230,000 new PCa cases are diagnosed in the US each year. Many PCas may remain indolent for the rest of the patients' lives [1]. Some of these PCas, however, may progress to metastatic disease, which is resistant to most common cancer therapies and is often lethal. Currently the PCa prognosis is based on Gleason Score (GS) [2] of the biopsied prostate tissues obtained routinely for PCa diagnosis/prognosis. While GS≥ 8 generally represents aggressive tumors, a large majority of patients have tumors with GS ≤7 with uncertain prognosis. Unfortunately, there is no clinically accepted method that can reliably identify the aggressive tumors in these patients. Therefore, more than 80 percent of these patients (>80,000/year) opt for major invasive therapies such as radical prostatectomy or ionizing radiation therapy with many suffering from side-effects such as incontinence, impotence, etc. A valid PCa prognostic indicator should spare the patients with indolent tumors from unnecessary suffering. Additionally, a reliable method of identifying patients with aggressive tumor should ease clinical trial design for new agents that are being developed for treatment of early stage PCa. This should also considerably reduce health care expenditure. Thus, distinguishing the aggressive from the indolent tumors is of paramount importance in PCa clinical management. Without a clear understanding of the mechanism of PCa progression, most current studies in PCa prognosis are focused on comparing genomic and proteomic analysis of tumor tissues and surrounding "normal" stroma [reviewed in 3]. Most of these studies focus on clinical validation of the presence/absence or expression/repression of certain marker genes. Without functional characteristics, the identified biomarkers cannot be mechanistically linked to the process of cancer progression, migration and metastasis and thus far are only partially successful in identifying aggressive tumors.

Accumulated evidence during the last 10+ years has shown that excess reactive oxygen species (ROS) play a critical role in PCa recurrence and progression to often-lethal castrate-resistant PCa (CRPCa) [4–9]. Multiple studies estimated ROS-induced hydroxylation and nitrosylation of cellular macromolecules in normal prostatic epithelia and PCa tissues. Pairs of cancer and normal tissues from the same PCa patient or from the same transgenic animal developing spontaneous PCa have been reported [8–10]. Data showed that the enzymes responsible for ROS production as well as ROS-induced macromolecular modifications are significantly higher in the PCa cells as compared to their normal epithelial counterparts both in men and in mice. A series of publications in the last several years have shown that at least one ROS, H_2O_2, plays a central role in certain autocrine growth factor expression in PCa cells that prevents apoptosis and sustains androgen-dependent PCa proliferation in the absence of androgen [11–16]. It has been shown that nitric oxide radical (NO) produced in oxidatively stressed cells may cause PCa invasion and migration [12,17]. Thus, identification of a metabolic pathway that can relate to high ROS production in PCa cells may help delineate a mechanism of PCa progression. Until recently, few such pathways that may lead to excess ROS production in any cancer cell have been reported.

We have reported that androgens cause upregulation of a transcription factor JunD, which associates with activated androgen receptor (AR) in human PCa cells [18–20]. The resultant protein complex induces gene expression and consequent increase in the enzymatic activity

of Spermidine/spermine N^1 acetyl transferase (SSAT) [21]. SSAT is the first enzyme that regulates a major spermidine/spermine (polyamine) oxidation pathway. It converts the spermidine and spermine to their corresponding acetyl derivatives. These products are oxidized by the enzyme acetyl polyamine oxidase (APAO) generating copious amounts of H_2O_2 in spermidine/spermine-rich PCa cells [22–26]. We have silenced SSAT expression by stably transfecting LNCaP cells with shRNA against SSAT (siSSAT) [21]. Unlike in the parental LNCaP cells, androgens fail to increase ROS in the siSSAT cells.. These results led us to ask if such a mechanism of ROS production is also active in human PCa and if it can be related to PCa progression and metastasis.

Here, we report the expression of SSAT mRNA and the enzyme protein in two human prostate tissue microarrays (TMA) consisting of various types of cancerous and non-cancerous prostate tissues obtained from human prostate biopsies and prostatectomy tissue sections. Our analysis, reported here, show a significant direct correlation between SSAT expression in PCa tissues and disease progression. Based on these and other published results, we propose one likely mechanism of PCa progression that can be exploited not only to identify patients with aggressive PCa, but can also be targeted for development of new anti-PCa agents for therapy of potentially progressive disease at an early-stage.

Methods

Study population

Two different patient cohorts were used under the IRB approval #: 2013-0920: The first cohort consists of 95 PCa patients with a median age of 62 years (range 43–82 yrs) and 24 patients with benign prostate hyperplasia (BPH) with a median age of 68 years (range 59–86 yrs). The archival prostate tissues in this cohort were collected from both transurethral resection of prostate (TURP, for BPH only) and prostatectomies performed from 1995 to 2006, from which a progression TMA (pTMA) was constructed. The pTMA consists of 384 duplicate cores from different disease groups: 43 localized PCa (PCa_local, pT2), 30 aggressive PCa (PCa_aggr, pT3 and 4), 22 metastatic PCa (Met, cancer outside of prostate), 25 high grade intraepithelial neoplasia (HGPIN) (tissue from HGPIN blocks of some of the PCa patients of this cohort) and 48 benign prostate tissues (BPT) (from the non-tumor blocks of some of the cancer patients in this cohort) and 24 BPH. The second cohort consists of 183 PCa patients with a minimum follow-up period of 5 years. The median age of 60.2 years (range 45–75 years) and a median Gleason score of 7 (range 4–9). The archival prostatectomy tissues were collected from 1999 to 2005, from which an outcome TMA (oTMA) was made and the patients were followed for disease outcome until this day. The oTMA consists of 462 duplicate cores from different disease groups: 183 prostate cancer (PCa) tissues (from 125 patients without recurrence, 38 with biochemical recurrence defined as elevated PSA following prostatectomy in the absence of clinical evidence of recurrence, and 20 with cancer recurrence either local or distant) and 48 BPT (from non-tumor blocks of some of the PCa patients in this cohort). Tissue characterisitics in these TMAs are summarized in Table 1. There were 11 disease-specific death events. The median recurrence-free survival and overall survival has not been reached after a median follow-up time of 10.6 years (range 5.0–14.6).

Immunohistochemistry (IHC)

SSAT protein expression levels were detected from one pTMA and one oTMA section with rabbit anti-human SSAT polyclonal antibody (Santa Cruz Biotechnology, Inc, sc-67159), visualized with multimer-horse radish peroxidase (HRP) and betazoid 3,3'-Diaminobenzidine (DAB) and counterstained with Hematoxylin using an autostainer (Discovery XT, Ventana Medical Systems, Inc., Tucson, AZ) following a published protocol [25].

RNA In situ hybridization (RNA-ISH)

SSAT mRNA expression levels were detected from one pTMA and one oTMA section with SSAT-specific probes and RNAscope detection kit following the manufacturer supplied protocol for RNAscope2.0 HD, brown (Advanced Cell Diagnostics (ACD), Hayward, CA). SSAT mRNA and protein expression levels were visualized with DAB and quantitated using an Automated Image Acquisition and Analysis system (see below).

Automated Image Acquisition and Analysis

The stained TMA slides were loaded onto the Vectra slide scanner. A scanning protocol was created based on the TMA core number and size. Nuance multispectral image cubes were acquired with 20x objective lens (0.5 micron/pixel) and using a full CCD frame at 1×1 binning (1,360×1,024 pixels) for analysis. Any core with less than 5% epithelial component or tissue folding or loss of tissue was excluded for analysis. Nuance3.0.2 software was used for building the spectral library of DAB and hematoxylin. The spectral library was then used to unmix the signals on the two-colored TMA slides by recognizing their unique spectral curves for quantitation. This method eliminated any signal cross-talk. InForm1.4.0 software was used to segment tissue compartments (epithelium vs. non-epithelium) and subcellular compartments (nucleus vs. cytoplasm). The target signals were quantitated within the selected tissue and subcellular compartment(s) of interest. SSAT protein and mRNA expression levels from each sample were quantitated as optical density (OD) per unit area (pixel) and the expression level was normalized to the total pixel counts obtained from each cell. SSAT mRNA is expressed in both cytoplasm and nucleus whereas SSAT protein is expressed only in the cytoplasm. Therefore, SSAT mRNA was quantified in the epithelial cells whereas SSAT protein was quantified in the cytoplasm.

Statistical Analysis

SSAT mRNA and protein expression levels were summarized in terms of means and standard errors. In order to account for correlations between duplicate cores from the same subjects, linear mixed effects modeling with subject specific random effects was conducted to compare SSAT mRNA and protein expression levels between tissue type groups. Dunnett's procedure was used to compare expression levels between the benign tissue type group to all other groups for the analysis of the pTMA, and to compare expression levels between the BPT tissue type group to all other outcome groups for the analysis of the oTMA. Tukey's Honestly Significant Difference (HSD) method was utilized when conducting all pairwise comparisons. Univariable and multivariable logistic regression analysis was conducted to evaluate whether SSAT RNA and protein expression levels

predict recurrence. Age at diagnosis, stage, lymph node status and Gleason score and treatment (hormonal vs. other) were included as covariates in the multivariable analysis. Odds ratios (OR) and the corresponding 95% confidence intervals (CI) were reported. Furthermore, the discriminatory power of SSAT protein expression for predicting recurrence was examined by conducting a Receiver Operating Characteristic (ROC) analysis. The Youden criterion was used to determine optimal threshold values of SSAT expression for predicting recurrence [27]. The association between SSAT expression and time to recurrence was evaluated by conducting univariable and multivariate Cox proportional hazard regression analysis. Time to recurrence was defined as the number of months from the date of tissue collection to the date of cancer recurrence or last date of follow-up. The results of these analyses were summarized in terms of hazard ratios (HR) and the corresponding 95% confidence intervals. All P values are two-sided and $P<0.05$ was used to determine statistical significance. Statistical analyses were conducted using SAS software version 9.2 (SAS Institute, Cary, NC).

Results

SSAT mRNA expression in Human PCa by RNA-*in situ* hybridization (RNA-ISH)

Our published data show that cultured human PCa cells express SSAT mRNA and demonstrate considerable increase in enzyme activity at normal serum androgen [21]. We asked if any human PCa tissue also expresses SSAT. First, we tested SSAT mRNA expression in the human prostate tissue sections in the TMAs. We used a recently commercialized RNA *in situ* hybridization (RNA-ISH) technique (see Methods for detail) capable of detecting and estimating specific mRNAs by unique sequence matching from the 300–1,000 base-long mRNA fragments present in the FFPE sections [28]. Representative RNA-ISH image obtained from the normal area of a PCa patient biopsy sample is shown in Figures 1B and the cancer area of the same biopsy sample is shown in Figure 1D. The corresponding H&E images generated from an adjacent section of the same tissue has been shown in Figures 1A and 1C, respectively. The images show a markedly higher SSAT mRNA expression in PCa than in normal prostatic epithelia. When the H&E images (Figures 1A and 1C) are compared with the SSAT staining, the data also show that most of the mRNA is sequestered in the nuclei of the cells marked by the Hematoxylin staining with little mRNA staining in the cytoplasm.

The DAB stain-intensities in pTMA and oTMA were quantitated using an image quantitation method standardized in our laboratory [29] and described in the Methods. The results are shown in Table 2 and Figure 2. The pTMA data show that the SSAT mRNA levels in the epithelial cells were significantly higher in all PCas as compared to the benign prostate tissue (BPT) ($p<0.005$). Statistical analysis showed that there was no significant difference in SSAT mRNA levels between BPH and the BPT ($p=0.514$). There was also no significant difference between the localized or aggressive PCa groups as well as between PCa and BPH ($p=0.743$ and $p=0.271$). There was no statistically significant difference detected in SSAT mRNA expression for the oTMA between clinical outcome groups as well. The mRNA levels, however, were significantly higher in the metastatic PCa group, when compared to all other groups in the pTMA ($p<0.021$).

Logistic regression analysis was conducted to evaluate the association between SSAT mRNA expression and cancer recurrence in the oTMA. The results are shown in Table 3. In this analysis, SSAT mRNA expression did not predict recurrence (unadjusted OR=1.14, 95% CI: 0.64–2.01). Furthermore, after adjusting for age at diagnosis, stage, lymph node status and Gleason Score, no statistically significant association between SSAT mRNA expression and time to recurrence (adjusted HR=1.10, 95% CI: 0.65–1.88) was observed.

SSAT protein expression in Human PCa by Immunohistochemistry (IHC)

Representative IHC images of the benign and cancerous prostatic epithelial cells are shown in Figures 1E and 1F, respectively. The image clearly demonstrates that the SSAT protein is nearly absent in benign prostatic lumen, while markedly high in the cytoplasm of the PCa tissues in the prostate section taken from the same patient.

The quantitation of the IHC images for SSAT protein in the cellular cytoplasm of the cells in the pTMA and oTMA was achieved following the same procedure as for the RNA-ISH data using anti-SSAT antibody (see Methods). These results are also shown in Table 2 and Figure 2. In the pTMA, the SSAT protein expression is significantly enhanced in the PCa tissues as compared to the benign and BPH tissues ($p<0.0001$). It is further enhanced in the aggressive and metastatic PCa as compared to localized tumor ($p=0.026$ for comparing metastatic versus localized tumor). In the oTMA, SSAT protein expression levels were significantly higher in PCa tissues as compared to tissues obtained from the benign prostatic regions of the same patients ($p<0.001$). Furthermore, a marginally significant difference in SSAT protein expression levels was also detected when comparing subjects with cancer recurrence (both biochemical as well as radiographic) to subjects with no recurrence ($p=0.052$). The odds ratio in the univariate logistic regression analysis of cancer recurrence on SSAT protein percent expression was 1.28 (95% CI: 0.92–1.78) and 1.31 (95% CI: 0.95–1.92) in the multivariate logistic regression analysis after adjusting for age at diagnosis, stage, lymph node status, Gleason Score and treatment (Table 3). The adjusted hazard ratio for predicting time to recurrence in the multivariate Cox proportional hazard regression analysis was 1.13 (95% CI: 0.81–1.56) (Table 3), while the adjusted hazard ratio for disease-specific survival was 2.20 (95% CI: 1.02–4.75) (data not shown). The area under the curve (AUC) for the Receiver Operating Characteristics curve was 0.66 (95% CI: 0.55–0.77), indicating a moderate level of discriminatory power of SSAT protein expression for predicting cancer recurrence. The optimal cut-off value for SSAT protein expression in cellular cytoplasm based on the Youden criterion was 0.045, resulting into a sensitivity of 0.61 (95% CI: 0.53–0.68) and a specificity of 0.67 (95% CI: 0.44–0.84).

Discussion

The data obtained from the pTMA presented in Figure 2A show that there is a significant increase in the both SSAT mRNA and the SSAT enzyme protein in the cell nuclei and cytoplasm, respectively, of PCa cells as compared to their benign counterpart. This increase is even more pronounced in metastatic PCa tissues as compared to the PCa localized in the prostate. In contrast, the data obtained from the oTMA presented in Figure 2B show that only the SSAT protein and not the mRNA level is significantly increased in patients, who

ultimately progress to advanced metastatic PCa. It is to be noted that the tissues in the pTMA includes biopsy tissues from patients with metastatic disease in addition to prostatectomy tissues from patients with localized PCa. The oTMA, however, consists only prostatectomy tissues from patients with early-stage disease. As mRNAs assemble into specific ribonucleoproteint complexes ((m)RNPs) before export to cytoplasm before being transcribed, it is possible that there are other yet unknown RNA processing/transporter proteins that also play critical role in PCa progression. Thus, while both SSAT mRNA and protein are positively correlated in metastatic PCa, some early-stage PCa expressing SSAT mRNA present in the oTMA may not progress due to a lack of these other factors that prevent mRNA transport/transcription to yield functional SSAT enzyme to produce the metabolites that lead to an increase in cellular ROS and PCa progression. Thus, while SSAT protein expression is correlated with PCa progression in both oTMA and pTMA, SSAT mRNA expression is only correlated with PCa progression in the pTMA.

It is known that ROS levels are higher in invading adenocarcinomas as compared to the normal epithelia [6–9]. It is also established that ROS induce activation of nuclear transcription factor-κB (NF-κB) [30,31]. The presence of both canonical and non-canonical components of activated NF-κB in the cell nuclei has been correlated to poor outcome of human prostate cancer [32,33]. In several human cancer cell lines including human PCa cells (our unpublished data), NF-κB induces SSAT gene expression [reviewed in 26]. Based on these results combined with the data presented here and also reviewed in [34] along with our published data [20,21], we propose an intriguing possibility of PCa progression that is shown schematically in Figure 3. In this scheme —a) activated androgen receptor-JunD complex induces SSAT expression; b) SSAT initiates enhanced polyamine oxidation that produces high ROS levels in certain PCa cells; c) ROS activate NF-κB; d) NF-κB, in turn, induces SSAT expression. Thus, an autocrine feed-forward loop consisting of SSAT-ROS-NF-κB-SSAT is activated that sustains ROS production and NF-κB activation in the absence of androgen. Activated NF-κB prevents apoptosis [reviewed in 30], produces more ROS by activating SSAT and may also activate AR [31,32]. Several publications have shown that ROS induce autocrine growth factor release, sustain cell growth [11–16] and help cellular invasion and metastasis [12,17]. Our unpublished data also show that a non-canonical pathway of NF-κB activated by the ROS induced DNA damage and consequent activation of canonical NF-κB [35] or some other yet unknown way can also activate AR in the absence of androgen (Meheraein-Ghomi *et al*, manuscript in preparation). This autocrine loop can lead to PCa proliferation in the absence of androgen leading to the progression of androgen-dependent PCa to castrate resistant PCa (CRPCa). It has also been clearly demonstrated that ROS induce several functional proteins that help metastasis [8,17]. Thus, ROS generated by the activation of spermidine/spermine oxidation pathway initiated by SSAT expression can activate more than one mechanism to help androgen-dependent PCa cell survival and promote CRPCa growth as well as its metastasis to distant organs.

The data shown in Figure 2B and Table 2 for the oTMA tissues containing PCa tissues from early-stage patients show that only the SSAT protein and not the SSAT mRNA expression is related to tumor progression. These early-stage patients have normal serum androgen level. We believe that at this stage, sufficient SSAT protein level and enzyme activity is

maintained by the androgen-JunD axis of SSAT activation without a marked increase in gene transcription leading to SSAT mRNA production. Thus, the cancer progression is dependent on the SSAT enzyme level/activity and the resultant increase in ROS. Much of the metastatic PCa tissues present in the pTMA, however, were derived from advanced patients, many of whom have undergone androgen-deprivation therapy (ADT) and have low serum androgen levels. According to our hypothesis, in these patients with CRPCa the cancer cells are producing ROS due to SSAT activation induced by activated NF-κB. Several publications have shown that NF-κB causes a strong induction of SSAT gene expression [reviewed in 26]. We believe that this induction pathway is stronger than that in the AR-JunD axis leading to an enhanced mRNA expression. The excess SSAT mRNA is sequestered in the nuclei (nucleoli) of the cells. Thus, the pTMA tissues show significant increase in both nuclear mRNA and the cytoplasmic enzyme protein in the progressed metastatic disease. ROS induction through SSAT enzyme induction also leads to the catabolism of spermidine and spermine, two known scavengers of free radicals. Thus, a decrease in these intracellular polyamines may also lead to further increase in cellular ROS.

The SSAT/APAO pathway of polyamine oxidation is mainly a peroxisomal oxidation phenomenon, which takes place in the presence of peroxisomal catalase and thus, may substantially attenuate the amount of H_2O_2 production. Since the amount of polyamines present in PCa cells is in the millimolar concentrations, the cellular catalase activity may not be enough to reduce all the H_2O_2 produced in this pathway. In addition, a recent publication has shown that C-262T single nucleotide polymorphism (SNP) in catalase gene that reduces catalase enzymatic activity [36] is directly correlated with PCa progression [37], further suggesting that the peroxisomal oxidation of polyamines through SSAT/APAO pathway may play a significant role in PCa progression

It is to be noted that another pathway of PCa progression in a transgenic mouse model has been shown. In that model, B-cell with activated NF-κB migrates into the inflammatory zone of the PCa region. It has been proposed that the activated NF-κB induces certain B-cell specific metabolic pathways that release paracrine factors to activate PCa progression [38]. Existence of such pathways in human prostate, however, has not yet been shown. Moreover, the canonical and non-canonical expressions of NF-κB proteins within the human PCa cells have been correlated with the disease outcome [32,33] suggesting a key role played by a cellular autocrine pathway activation in the PCa cells. SSAT induced cytoplasmic H_2O_2, however, can be released in the stroma and induce inflammatory response and B-cell infiltration. Thus, a role of SSAT induction leading to B-cell infiltration and related stromal changes for PCa progression can also be conjectured.

From our results and published data from other laboratories, we conclude that SSAT induction and the resultant ROS production play a key role in PCa progression and metastasis at least through an autocrine feed-forward pathway and may also induce a paracrine signaling. Thus, SSAT enzyme induced pathways can be exploited to predict disease outcome, identify patient cohort for clinical trials of early-stage anti-PCa agents as well as further development of future targeted therapy.

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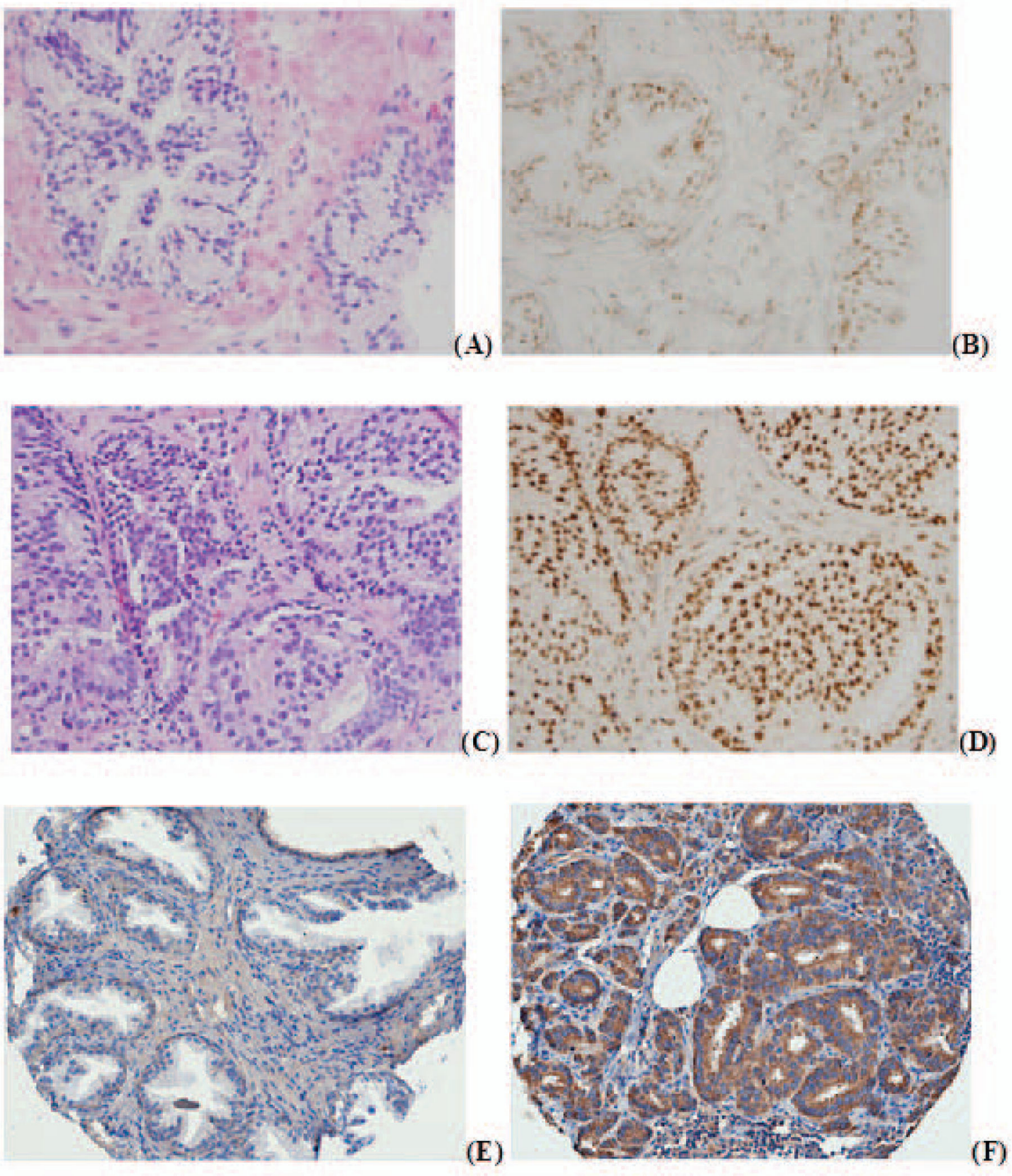

Figure 1.

H&E (A&C), SSAT mRNA (B&D) patient biopsy tissues stained by RNA-ISH containing PCa (C&D) and normal epithelium (A&B) and SSAT enzyme protein in BPT (E) and in PCa (F) stained by immunohistochemistry using anti-SSAT antibody.

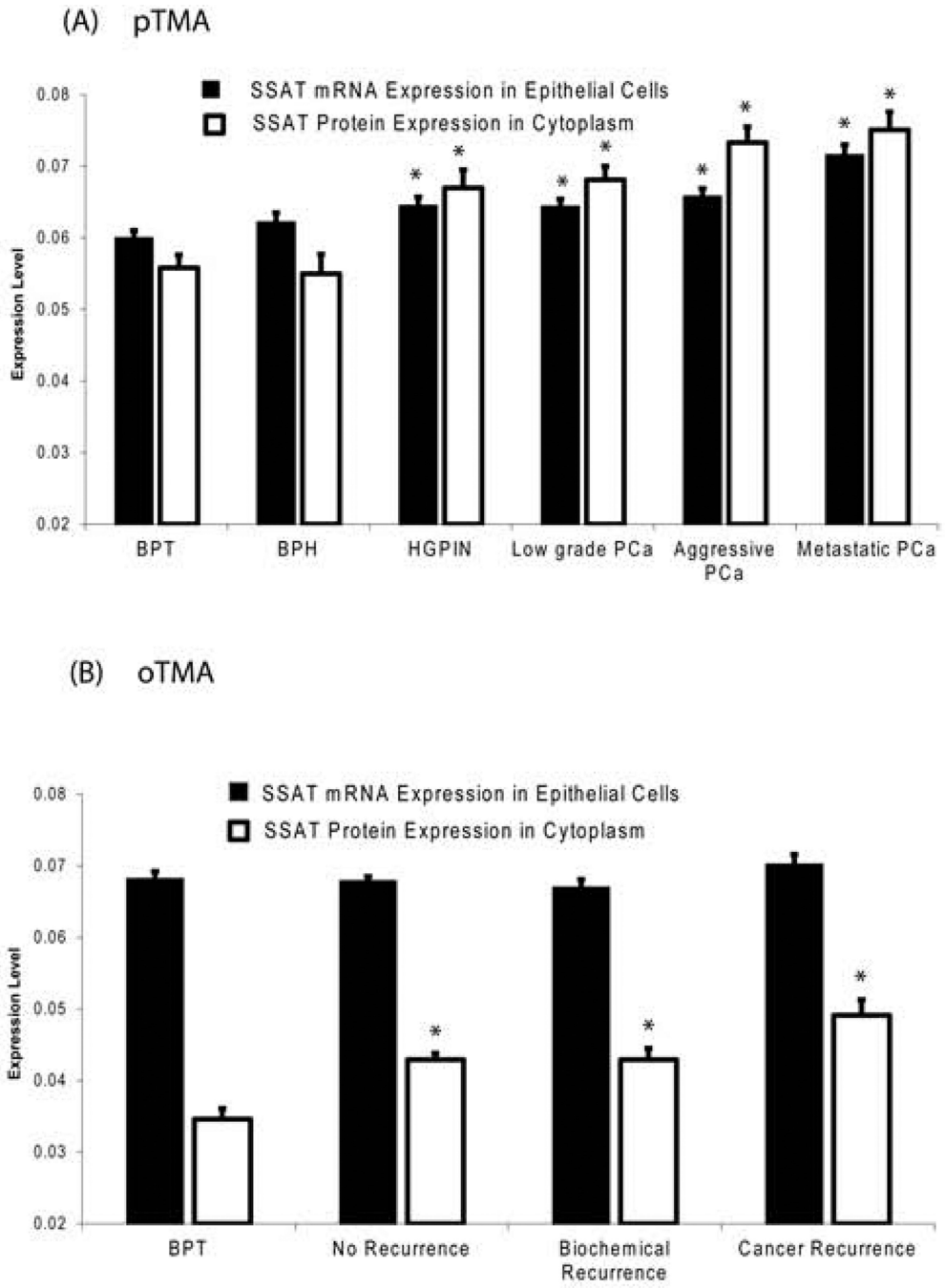

Figure 2.

SSAT mRNA expression in epithelial cell nuclei and SSAT protein expression levels in the cellular cytoplasm for (A) pTMA and (B) oTMA.

* indicates a statistically significant difference, when compared with the benign group.

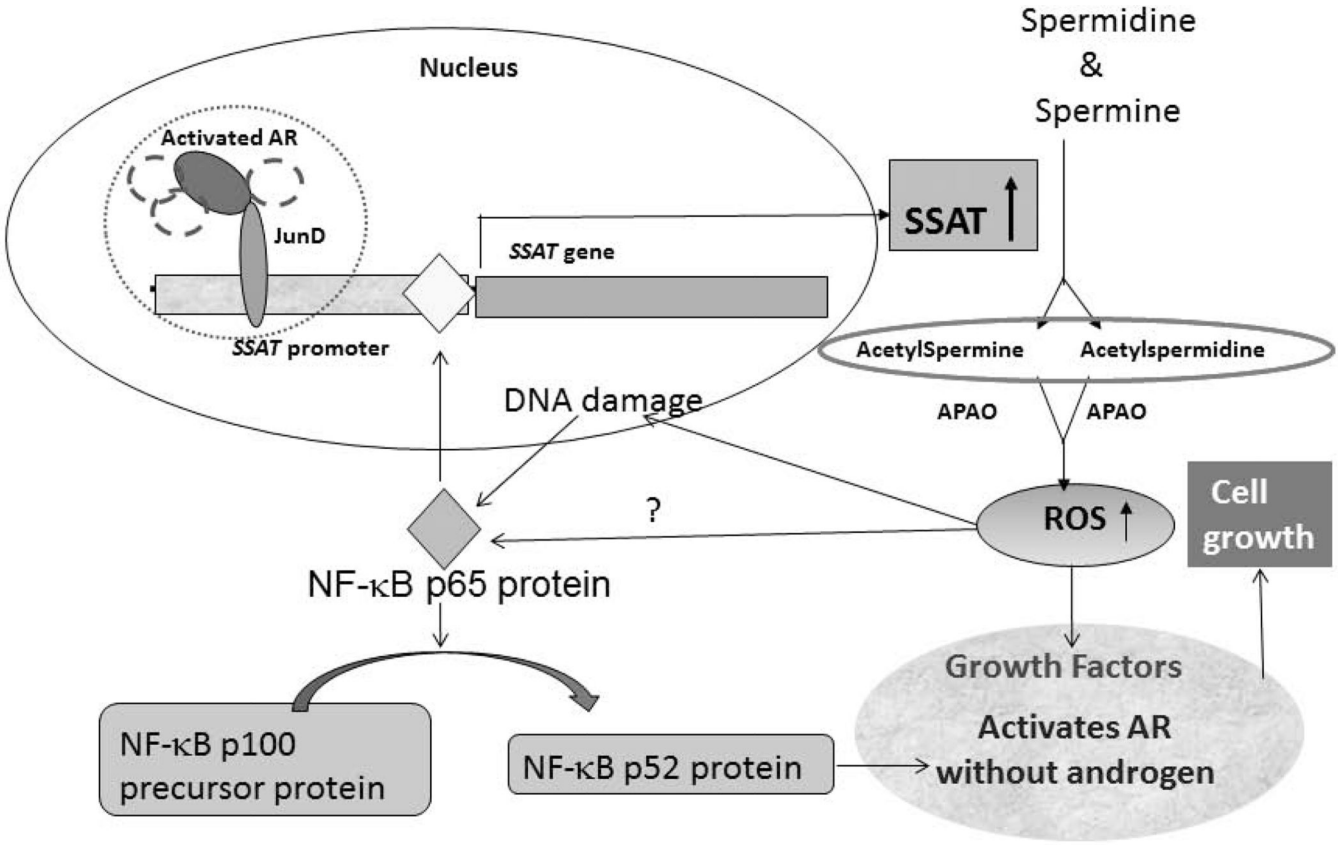

Figure 3.
A mechanism of high ROS (H_2O_2) induced PCa growth in the absence of androgen

Table 1

Tissue number and patient age in the TMAs

TMA (cohort)	Disease Status (#core)	Median age (y) (Range)
pTMA (cohort 1)	BPH (48)	68 (59–86)
	BPT (96)	62 (37–74)
	HGPIN (50)	64 (54–81)
	PCa_local (86)	57 (43–74)
	PCa_aggr (60)	64 (43–69)
	Met (44)	69 (51–82)
	Disease Recurrence (#core)	**Median age (y) (Range)**
oTMA (cohort 2)	None (250)	60 (45–75)
	Biochemical (76)	62 (49–71)
	Cancer (40)	57 (48–68)

Table 2

SSAT mRNA and protein expression levels in TMAs

			SSAT mRNA expression levels in epithelial cells			SSAT protein expression levels in cytoplasm		
	Group	**N**[†]	**Mean**	**SE**	**p-value**[‡]	**Mean**	**SE**	**p-value**[‡]
pTMA	**Benign**	96	0.0600	0.0010		0.0558	0.0018	
	BPH	48	0.0622	0.0013	0.5140	0.0550	0.0027	0.9994
	HGPIN	50	0.0645	0.0012	0.0142	0.0670	0.0025	0.0015
	Low grade PCa	86	0.0644	0.0010	0.0049	0.0681	0.0019	<0.0001
	Aggressive PCa	60	0.0658	0.0011	0.0003	0.0733	0.0022	<0.0001
	Metastatic PCa	44	0.0716	0.0014	<0.0001	0.0751	0.0025	<0.0001
oTMA	**BPT**	96	0.0682	0.0010		0.0346	0.0015	
	No Recurrence	250	0.0679	0.0006	0.9840	0.0429	0.0009	<0.0001
	Biochemical Recurrence	76	0.0670	0.0011	0.7753	0.0429	0.0016	0.0007
	Cancer Recurrence	40	0.0702	0.0014	0.5088	0.0491	0.0022	<0.0001

[†]Number of duplicate cores per group

[‡]Comparison to benign group (adjusted using Dunnett's method)

Table 3

SSAT expression as predictors for 5-year recurrence rate and time to recurrence

Group	OR† (95% CI)	OR‡ (95% CI)	HR^ (95% CI)	HR^^ (95% CI)
SSAT mRNA expression levels (%) in epithelial cells	1.14 (0.64–2.01)	1.06 (0.55–2.07)	1.04 (0.61–1.76)	1.02 (0.56–1.81)
SSAT protein expression levels (%) in cytoplasm	1.28 (0.92–1.78)	1.31 (0.89–1.92)	1.20 (0.89–1.63)	1.13 (0.81–1.56)

† Unadjusted odds ratio for predicting cancer recurrence

‡ Adjusted (by age, stage, lymph node status, Gleason score and treatment) odds ratio for predicting cancer recurrence

^ Unadjusted hazard ratio for predicting time to cancer recurrence (E=20 recurrence events)

^^ Adjusted (by age, stage, lymph node status, Gleason score and treatment) hazard ratio for predicting time to cancer recurrence (E=20 recurrence events)