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Clinical implications of AGR2 in primary prostate cancer: Results from a large-scale study

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Human anterior gradient-2 (AGR2) has been implicated in carcinogenesis of various solid tumours, but the expression data in prostate cancer are contradictory regarding its prognostic value. The objective of this study is to evaluate the expression of AGR2 in a large prostate cancer cohort and to correlate it with clinicopathological data. AGR2 protein expression was analysed immunohistochemically in 1023 well-characterized prostate cancer samples with a validated antibody. AGR2 expression levels in carcinomas were compared with matched tissue samples of adjacent normal glands. AGR2 expression levels were dichotomized and tested for statistical significance. Increased AGR2 expression was found in 93.5% of prostate cancer cases. AGR2 levels were significantly higher in prostate cancer compared with normal prostate tissue. A gradual loss of AGR2 expression was associated with increasing tumour grade (ISUP), and AGR2 expression is inversely related to patient survival, however, multivariable significance is not achieved. AGR2 is clearly upregulated in the majority of prostate cancer cases, yet a true diagnostic value appears unlikely. In spite of the negative correlation of AGR2 expression with increasing tumour grade, no independent prognostic significance was found in this large-scale study.

Key words: AGR2; prostate cancer; immunohistochemistry; TMA; androgen receptor.

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In 2020, prostatic cancer (PCa) was the most frequent cancer among men with more than 1.4 million new cases as well as the fifth leading cause of cancer death among men [1]. Still, due to the fairly indolent nature of this neoplasm, most patients diagnosed with prostate cancer will not die from it. However, a certain population will eventually succumb, which is socio-economically and epidemiologically relevant due to the high frequency of the disease with incidence rates expected to continuously increase over the next years [2]. Therefore, it is of major importance to delineate these groups of patients and to provide individual information concerning the prognosis of each patient. If possible, this would allow a well-founded decision on the various therapeutic options available for this disease. Tumour grade (Gleason score [GS]/ISUP grade groups), TNM staging, prostate-specific antigen (PSA) levels and tumour margin status are well established for the prognostic stratification of

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prostate cancers [3–5]. However, patients with comparable histological and clinical prostate cancer parameters can demonstrate strikingly varied presentations of clinical outcomes [3]. Therefore, prognostic biomarkers that add information about prognosis or even therapeutic prediction are highly warranted.

A candidate biomarker for prostate cancer, others and we identified in former studies, is Anterior Gradient 2 protein (AGR2) [4–9]. This is a hormone-inducible secreted protein localized on chromosome 7p21 that was first identified as an orthologue of the Xenopus laevis gene *XAG*-2 [5]. It is a member of the protein disulphide isomerase family of endoplasmic reticulum-resident proteins, exhibiting basic features of pro-oncogenic proteins in humans [10–12]. Nonetheless, several studies have documented the role of AGR2 in physiological as well as pathological processes [10–13]. Generally, endoplasmic reticulum stress is seen as an inducer of AGR2 production through unfolded protein response (UPR) [10, 13] and it has been shown that AGR2 can inhibit the function of p53, which gives it a possible pro-oncogenic function [10, 12].

In humans, AGR2 expression has been reported in various adenocarcinomas and other tumours (reviewed in [14]). While most of the published studies have focused on the role of AGR2 in breast and prostate cancer, it has also been described in malignant tumours of the lung, liver, ovaries, intestine and urinary bladder [15–20]. In most of these entities, AGR2 was found upregulated in comparison to normal tissue. Several studies have demonstrated a correlation between increased AGR2 expression and poor prognosis in lung, breast and gastric cancer [14, 20–26]. Controversially, some studies discovered a protective effect of AGR2 upregulation on tumour progression [15, 16, 27]. In breast cancer, AGR2 expression is associated with estrogen receptor positivity and it is thought to enhance metastasis in the subgroups of estrogen receptor-positive and tamoxifen-treated tumours [20, 28]. Most studies advocated a pro-invasive, pro-metastatic or adverse prognostic effect of AGR2 over-expression in the respective tumour.

In an earlier study, we found AGR2 overexpressed in 89% of prostate carcinomas, but no prognostic value [7]. Since, numerous studies have confirmed the overexpression of AGR2 in prostate cancer [4–6, 8, 29–31]. The prognostic value of AGR2 expression in prostate cancer remains controversial to date, as some studies reported no prognostic value, while others reported a better or a worse outcome of AGR2-positive tumours. These conflicting findings prompted us to clarify the prognostic value of AGR2 in prostate cancer by a large-scale expression analysis of representative prostate cancer cohorts following radical prostatectomy.

MATERIALS AND METHODS

Patients

In order to evaluate the expression of AGR2 in prostate cancer, three previously published cohorts of radical prostatectomy (RP) cases were used. One cohort consisted of 640 prostate cancer patients, who underwent RP between 1999 and 2005 at the Charité University Hospital in Berlin, Germany [7, 32]. The next cohort consisted of 238 patients having undergone RP for treatment of primary prostate cancer between 1999 and 2006 at University Hospital Zurich, Switzerland [33]. The last cohort consisted of 300 patients, who underwent RP at the University Hospital Bonn between 2000 and 2008 [34]. All cohorts were in tissue micro-array format, as described. We restricted the analysis to patients with clinical follow-up data, leaving 1023 patients in the analysis. The clinical-pathological parameters are given in Table 1. In this cohort, 212 patients (20.7%) experienced a biochemical recurrence (BCR), defined as a rising PSA value >0.2 ng/mL, after a median time of 50 months. BCR was used as an endpoint in the survival analyses.

The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Appropriate ethic approvals have been obtained before the study begin from the respective local ethics committees (Berlin: EA1/06/2004; Zurich: StV 25-2007; Bonn: 071/14).

Cell culture

PC-3 (ATCC, Manassas, VA) were cultivated in Ham's F-12 Medium/Kaighn's Modification, supplemented with 10% fetal bovine serum (FBS) (all cell culture media and supplements Invitrogen, Carlsbad, CA). Cells were cultured at 37°C, 5% CO_2 and 100% humidity.

Cell lysis and Western blot analysis

Cells were lysed in 60 mM n-Octyl-ß-D-glucopyranoside (Sigma-Aldrich, St. Louis, MO) in the presence of protease inhibitors (complete, Mini, EDTA-free, Protease Inhibitor cocktail tablets; Roche Applied Science). Twenty micrograms of cleared lysates were separated by SDS-PAGE and transferred to a polyvinylidene fluoride membrane (Bio-Rad, Hercules, CA, USA). After blocking with 1% BSA in PBS-Tween20, membranes were probed with primary antibody (AGR2, clone 1C3, Abnova Corp., Taipei City, Taiwan; dilution 1:1000) followed by horseradish peroxidase-conjugated anti-mouse secondary antibody (Pierce Biotechnology, Rockford, IL, USA) incubation and detection via SuperSignal West Dura Extended Duration Substrate (Pierce Biotechnology, Waltham, MA, USA).

AGR2-siRNA transfection for transient gene knockdown

PC-3 cells were transfected using Lipofectamine (Invitrogen, Waltham, MA, USA) as a transfection reagent. Cells

Table 1. Clinical–Pathological parameters of the patients analysed

Variable	Number of patients	AGR2_low (%)	AGR2_high (%)	*p*-Value
All cases	1023	388 (37.9)	635 (62.1)	
Patient age at RP 62 (median 63) years				
≤63 years	521	174 (33.4)	347 (66.6)	0.003
>63 years	502	213 (42.5)	288 (57.5)	
pT status				
pT2	675	244 (36.1)	431 (63.9)	n.s.
pT3/T4	348	144 (41.4)	204 (58.6)	
R status				
R0	697[1]	242 (34.7)	455 (65.3)	0.002
R1	319[1]	143 (44.8)	176 (55.2)	
Tumour grading				
ISUP1	355[1]	129 (36.3)	226 (63.7)	0.011
ISUP2	331[1]	106 (32)	225 (68)	
ISUP3	130[1]	59 (45.4)	71 (54.6)	
ISUP4	111[1]	46 (41.4)	65 (58.6)	
ISUP5	90[1]	43 (47.8)	47 (52.2)	
PSA mean 10.9 (median 7.6) ng/mL				
<7.6 ng/mL	508[1]	179 (35.2)	329 (64.8)	n.s.
>7.6 ng/mL	489[1]	194 (39.7)	295 (60.3)	

[1]Missing data.

were transfected on the day of seeding with a final siRNA concentration of 10 nM (all siRNAs were purchased from Qiagen, Hilden, Germany). One day after transfection, the medium of PC-3 cells was changed. Knockdown efficiency was confirmed on the protein level using Western blot analysis.

Immunohistochemistry

Immunohistochemistry was carried out using freshly cut (3 µm) sections that were mounted on super frost slides (Menzel-Gläser, Braunschweig, Germany). The AGR2/Gob-4 monoclonal mouse antibody (clone 1C3, dilution 1:500) was incubated using BondMax autostainers (Leica Microsystems, Wetzlar, Germany. Pretreatment Protocol H2-60, detection with Refine DAB Polymer-Kit). Finally, the slides were briefly counterstained with hematoxylin, dehydrated and mounted. For the peptide blocking experiment, one duplicate slide was incubated with (a) primary antibody and (b) primary antibody that had been pre-incubated with a 10 M excess of the respective immunogenic AGR2 peptide (Abnova Corp., Taipei City, Taiwan). As negative controls, four slides were processed without primary antibodies.

Evaluation of immunohistochemical stainings

Immunostaining of AGR2 was evaluated by two pathologists (MM, GK) using a four-tier intensity scoring system (0 negative, 1 weak, 2 moderate and 3 strong). In order to enhance intra- and interobserver conformity, a panel with four representative pictures with different expression levels as a benchmark was used (Fig. S1).

Analysis of TCGA and GTEx data sets

An in silico analysis of AGR2 mRNA expression levels from publicly available transcriptome databases [The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx), prostate cancer cohort] in the Xena browser software was used to complement the immunohistochemistry data [35]. The endpoint used for survival analysis was biochemical recurrence (BCR).

Statistics

Statistical analysis (chi-squared tests, Fisher exact tests, Spearman correlation, Log-rank tests, Cox multivariate regression) was performed using SPSS 27 (SPSS Inc, Chicago, IL, USA).

Patients were dichotomized by the median expression of AGR2. Survival curves were visualized using the Kaplan–Meier plot with the difference between survival distributions assessed by the log-rank test. The Cox proportional hazards model was used to test the statistical significance of clinicopathological parameters in both a univariate and a multivariate setting. p Values of <0.05 were considered statistically significant.

RESULTS

Verifying specificity of the anti-AGR2 antibody (clone 1C3)

AGR2 expression on protein level was determined in PC-3 cells. A knockdown protocol based on RNA interference was established. Western blot analysis 72-h post-transfection showed a potent AGR2 knockdown on protein level with two different siRNAs, whereas one siRNA showed only a minor effect and one siRNA failed (Fig. 1A). Additionally, pre-incubation of the AGR2 antibody with an excess of its immunogenic peptide abolished immunoreactivity to background levels (Fig. 1B).

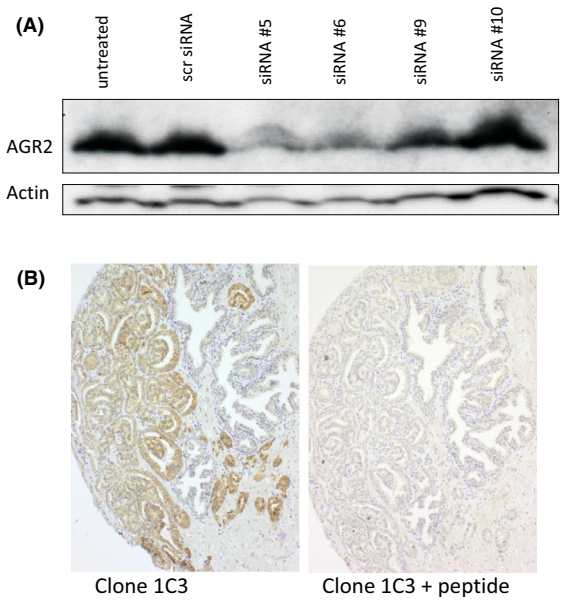

Fig. 1. Demonstration of AGR2 antibody specificity. (A) Transient knockdown of AGR2 by siRNA is seen in this Western blot for #5 and #6. (B) AGR2 Immunohistochemistry, demonstrating a predominantly cytoplasmic reaction in invasive epithelium (left). After pre-incubation with a 10 M excess of the immunogenic peptide, the immunoreactivity was reduced to background levels (right).

Staining patterns and expression levels of AGR2 in prostate tissues

AGR2 immunoreactivity was detected explicitly in the cytoplasm of secretory prostatic cells, no nuclear or membranous staining was noted. Connective tissue cells, blood vessels and lymphocytes were consistently negative. Benign normal tissue was predominantly AGR2 negative (Fig. 2A), however, atrophic benign epithelium was commonly AGR2 positive (Fig. 2B).

Sixty-six cancer cases were negative for AGR2 staining (score 0, Fig. 2C), 322 tumours showed weak staining (score 1, Fig. 2D), 374 carcinomas demonstrated moderate staining (score 2, Fig. 2E) and 261 tumours displayed a strong immunoreactivity (score 3, Fig. 2F). For statistics, AGR2 expression was dichotomized in either 'AGR2_low' (score 0 and 1; 37.9%) or 'AGR2_high' (score 2 and 3; 62.1%).

Association of AGR2 expression with clinical–pathological parameters

AGR2 expression in tumour tissues was significantly associated with younger patient age, negative margin status and lower ISUP grade groups. There was no significant association between AGR2 expression and tumour stage (pT-status) or PSA levels (Table 1). The associations with age and margins were confirmed in Spearman rank correlation analyses: Age: correlation coefficient (CC) −0.132, $p = 0.001$; Margins (R status) CC −0.101, $p = 0.001$. ISUP grade groups though dropped out (CC −0.049, $p = 0.120$), which clarifies that this association is weak.

Analysis of the prognostic value of low-level AGR2-mRNA using TCGA/GTEx databases

Using the TCGA/GTEx RNA-seq data sets, we analysed the association between low/high mRNA levels of AGR2 or AGR2-splice variants in an independent virtual cohort of 497 PCa patients. A normal tissue cohort of 100 samples (GTEx) was used as a control. There was a statistically highly significant association between reduced mRNA levels of AGR2-203, the predominant AGR2 isoform (major allele/wild type) and reduced patient survival ($p = 0.0223$). The splice variants AGR2-201 and AGR2-204 showed a similar tendency, however, the correlation remained statistically insignificant ($p = 0.0599$ and $p = 0.0668$, respectively) (Fig. 3B, C). AGR2-202 showed a significant correlation between high mRNA levels and a reduced progression-free interval ($p = 0.0464$) (Fig. 3D). AGR2-205, AGR2-206 and AGR2-207 were not studied, as they represent non-protein-coding AGR2 splice variants and in consequence do not affect IHC.

Prognostic value of clinicopathological parameters and AGR2 protein expression

First, univariate Cox analyses of the entire cohort were performed to demonstrate the prognostic value of clinicopathological parameters and hence confirm the representativity of this cohort. As expected, serum PSA, ISUP grade groups, pT categories and margin status could be verified as prognostic parameters in this cohort. Also, AGR2 expression in prostate carcinomas was demonstrated as a prognostic parameter, with tumours with lower levels showing an earlier biochemical disease progression (Table 2).

Additionally, survival curves were visualized using the Kaplan–Meier method, which confirmed the univariate prognostic value of AGR2 ($p = 0.015$, log-rank test) (Fig. 4A).

In a multivariate Cox regression, the conventional prognostic parameters remained highly significant, while AGR2 expression dropped out (Table 3).

In a Kaplan–Meier analysis stratified for ISUP grade groups (low grade = 1–2 vs. high grade = 3–5),

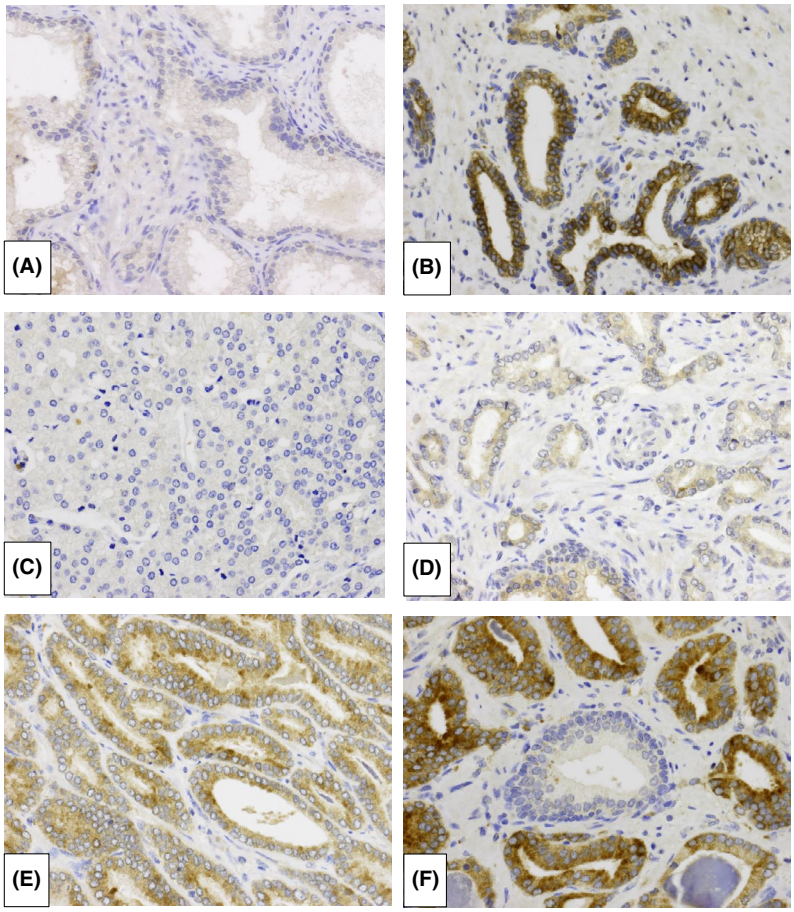

Fig. 2. AGR2 Immunohistochemistry in prostate tissues. (A) AGR2 is only very weakly expressed in secretory epithelium of benign glands. (B) Strong AGR2 staining was seen in some cases of atrophic epithelium. (C) Prostate carcinoma without AGR2 expression. (D–F) Prostate cancer with weak (D), moderate (E) or strong (F) AGR2 positivity. Note the AGR2-negative benign gland in the centre of (F).

AGR2 expression showed no separation of curves whatsoever in low-grade tumours (Fig. 4B), whereas in high-grade tumours, an AGR2 loss indicated a clear trend towards earlier biochemical relapse, but failed statistical significance (Fig. 4C, $p = 0.104$).

DISCUSSION

The protein AGR2 is a member of the protein disulfide isomerase (PDI) family and represents the human orthologue of the *Xenopus laevis* cement gland protein (XAG2) [36–38]. According to the Human Protein Atlas, higher protein expression rates are seen physiologically in tissues of the respiratory tract, gastrointestinal tract including the gallbladder and pancreas, the urinary bladder and genital organs of both sexes including endometrium, cervix, fallopian tube, placenta, prostate, epididymis and seminal vesicle (https://www.proteinatlas.org/ENSG00000106541-AGR2/tissue) [39]. The high expression seen in these organs indicates an involvement of AGR2 in mucus secretion but the functions of AGR2 appear to be much more complex. It plays a role in multiple signalling pathways including cell signalling, protein trafficking, proteostasis and in tumour chemoresistance and tumour cell dissemination [40].

Dysregulation of AGR2 expression and/or function is widely implicated in human diseases. Preliminary investigations suggest that downregulation or loss of function of AGR2 is involved in inflammatory bowel disease, ulcerative colitis and Crohn's disease [41–43]. Interestingly, AGR2 protein is often overexpressed and aberrantly secreted in a broad range of solid tumours [22]. An overview of studies analysing the prognostic value of AGR2 in solid tumours is given in Table 4.

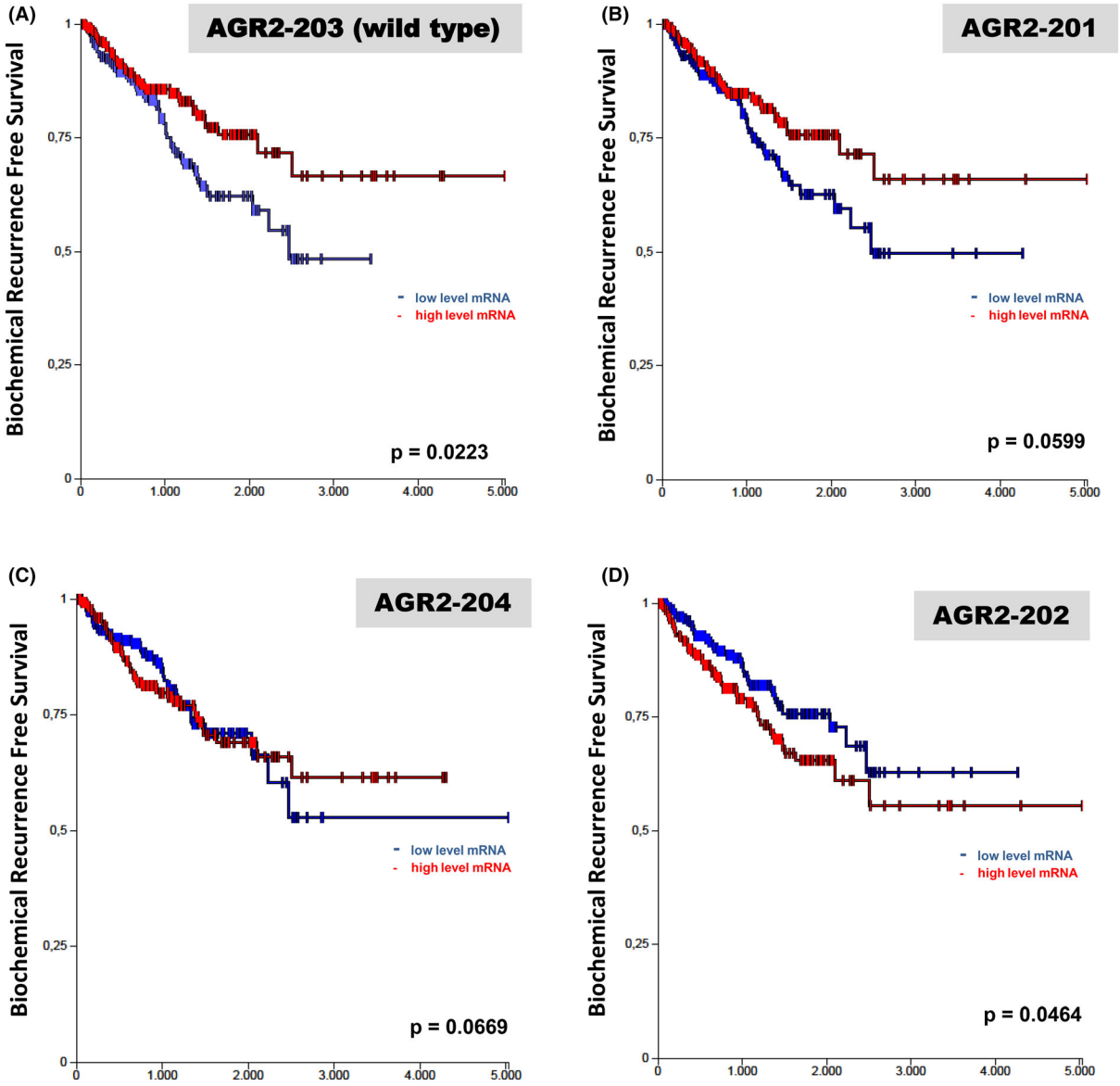

Fig. 3. Tumour sample data from TCGA/GTEx were dichotomized by median AGR2 expression and subjected to Kaplan–Meier analysis. (A) Kaplan–Meier curves for the predominant AGR2 isoform AGR2-203 (AGR2 wild type/major allele). Kaplan–Meier curves for AGR2 isoforms with known translation products (B) AGR2-201 (C) AGR2-204 and (D) AGR2-202.

There is experimental evidence that ARG2 can exert pro-oncogenic properties related to differentiation, migration/metastasis, proliferation, senescence, and chemoresistance when overexpressed or misplaced in the tumour niche/microenvironment, thus representing putative pro-oncogenic signalling intermediate in human cancer [9–11, 14, 44]. Although in vitro studies reveal a complex functional role of AGR2 in tumourigenesis and tumour progression, its prognostic or clinical significance remains controversial for most tumour entities. Aiming to clarify the prognostic value of AGR2 in PCa, a large-scale expression analysis of 3 representative prostate cancer cohorts with a total of 1023 patients was performed. Benign normal tissue was predominantly AGR2 negative, whereas the vast majority of prostate cancer cases were AGR2 positive, only 6.4% of the tumours were completely AGR2 negative. In tumour tissue, AGR2 expression was significantly associated with younger patient age, negative margins and lower ISUP grade groups. There was no significant association

Table 2. Univariate Cox regression: prognostic value of clinicopathological parameters (BCR as endpoint)

Variable	Hazard ratio	*p*-Value
Age (medianized)	1.094	n.s.
Serum PSA (medianized)	2.084	0.001
pT status (pT2 vs. pT3)	3.360	0.001
R status (R0 vs. R1)	2.911	0.001
Tumour grade (ISUP-grade groups)	1.882	0.003
AGR2 (medianized)	0.715	0.015

between AGR2 expression and tumour stage (pT status) or PSA levels. The prevalence of AGR2 in PCa compared with normal adjacent tissue is in agreement with previous studies in prostate cancer and other tumours [4, 5, 18, 30].

Initial studies investigating the prognostic value of AGR2 expression in PCa yielded conflicting results. While Zhang et al. [8] associated increased AGR2 expression in PCa with high GS and lower overall survival, a study by Maresh et al. [4] demonstrated earlier PSA relapses in tumours with low AGR2 expression. This trend became significant in a small group (65/187) of patients with advanced stage III/IV tumours. While the Gleason score, preoperative PSA and pT stage were good prognosticators in the unrestricted cohort of 187 patients, only pT stage remained significant in the restricted cohort, questioning the relevance of this subgroup. The discrepancy of the studies mentioned above could arise from the use of different antibodies and highlights the necessity of thorough antibody controls (Western blotting, protein knockdown, peptide blocking experiments) to exclude unspecific binding. Although we can rule out unspecific binding of our antibody (Fig. 1), we do not know the precise epitope recognized by it. Several studies revealed the presence of AGR2 splice variants in a number of tumours, including prostate cancer [11, 45, 46]. Without precise knowledge of the epitope recognized by the antibody, thorough discrimination of the various AGR2 forms is not possible and can affect the interpretation of IHC staining.

The absence of AGR2 immunoreactivity was observed in only 6.4% of PCa. To investigate a potential prognostic value of our immunohistochemical AGR2 data, tumour samples were dichotomized by the median expression of AGR2. Using Kaplan–Meier analysis, we found a significant association between reduced AGR2 expression and shortened patient survival ($p = 0.015$). This observation is consistent with several studies describing a gradual loss of AGR2 with increasing Gleason scores [6, 19, 30]. Though it is tempting to speculate, that the negative prognostic value of AGR2

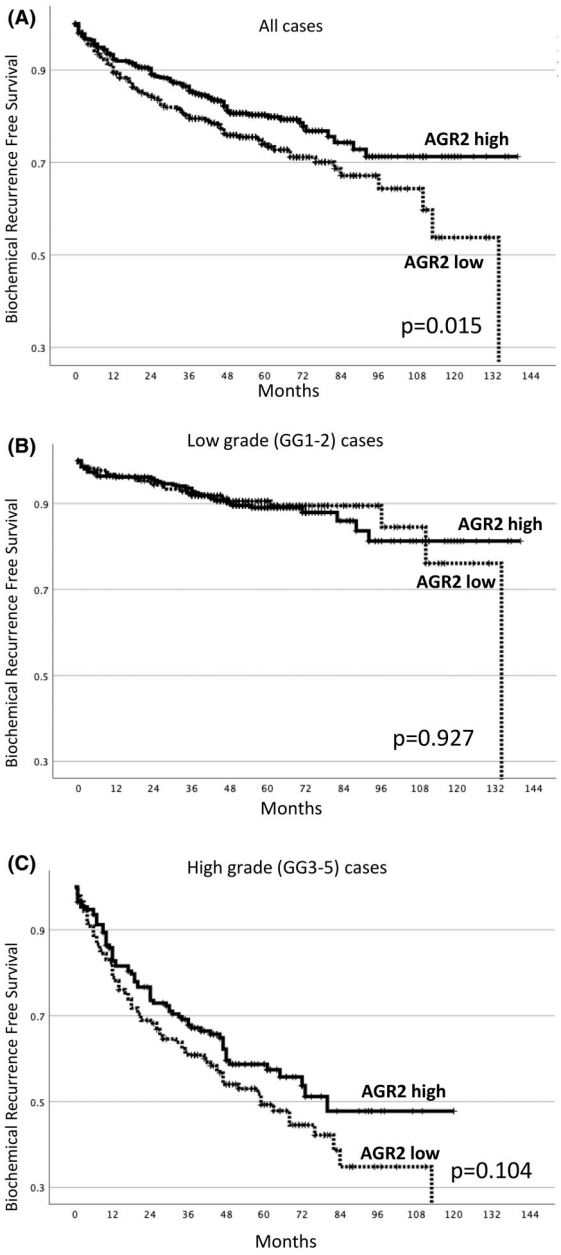

Fig. 4. Kaplan–Meier estimated biochemical recurrence-free survival curves according to AGR2 expression for 1023 patients with available biochemical follow-up data. (A) All cases. (B) Low-grade (GG1-2) cases. (C) High-grade (GG > 2) cases.

expression may be attributed to this correlation with tumour grade, we found in an analysis of AGR2 stratified for Gleason grade groups no prognostic value at all in low grade (GG1-2) tumour but a clear trend towards a prognostic value of AGR2 in higher-grade (GG3-5) tumours. This indicates, that the prognostic value of AGR2 in

Table 3. Multivariate Cox regression: prognostic value of clinicopathological parameters (BCR as endpoint)

Variable	Hazard ratio	*p*-Value
Serum PSA (medianized)	1.324	n.s.
pT status (pT2 vs. pT3)	1.595	0.005
R status (R0 vs. R1)	1.657	0.001
Tumour grade (ISUP)	1.649	0.001
AGR2 (low vs. high)	0.850	n.s.

prostate cancer, though it is expressed at lower levels in higher-grade tumours, is independent of tumour grade. In addition, we demonstrated an association between reduced AGR2-203 mRNA (AGR2 wild type) and lower patient survival in an independent virtual TCGA/GTEx-cohort of more than 490 PCa patients thereby confirming our IHC data (Fig. 3). As AGR2-202 is only weakly expressed across different cancers its putative protein is not expected to dramatically affect AGR2-203/major allele staining [11]. Unfortunately, IHC studies on ARG2 are still hampered by our limited knowledge of the role of AGR2 splice products. Of particular interest, the mRNA of a new splice variant, designated AGR2 SV-H, was able to discriminate between benign and PCa in tissue samples and

Table 4. Overview of AGR2 studies in solid tumours

Year	Author; PMID	Cancer type	Total cases	AGR2 in tumour (vs. benign)	Prognostic value of AGR2 expression; poorer/better survival	Particular findings
2018	Rodríguez-Blanco et al.; 30,559,929	Prostate	481	Up	Yes. Poorer.	High GS = ↓ AGR2 PSA n.s.
2013	Ho et al.; 23,348,903	Prostate	23 (248 cores)	Up	Yes. Better.	High GS = ↓ AGR2
2013	Kani et al.; 22,911,164	Prostate	44	Up	Not explored.	AGR2 in plasma associated with neuroendocrine differentiation
2011	Bu et al.; 20,945,500	Prostate	29 (tissues sections from RP)	Up	Not explored.	Low GS = ↑ AGR2
2010	Maresh et al.; 21,144,054	Prostate	187	Up	Yes. Better.	High GS = ↓ AGR2
2009	Pascal et al.; 20,021,671	Prostate	5	Up	Not explored.	High GS = ↓ AGR2
2007	Zhang et al.; 17,457,305	Prostate	106	↑ up (95.4% positivity)	Yes. Poorer.	↑ AGR2 significantly correlated with high GS and PSA levels
2005	Kristiansen et al.; 15,532,095	Prostate	91	Up (89% positivity)	No.	
2005	Zhang et al.; 15,834,940	Prostate	12	Up	Not explored.	n.s. correlation with age or Gleason score
2012	Darb-Esfahani et al.	Ovarian	124	Up (32% positivity)	Yes. Poorer.	
2006	Fritzsche et al.; 16,551,856	Breast	155	Up	Yes. Better.	
2009	Barraclough et al.; 19,834,055	Breast	315	up	Yes. Poorer.	
2018	Ann et al.; 29,796,176	Breast	1341	Up	Yes. Poorer.	
2009	Riener et al.; 19,609,859	Pancreas	148	Up	No.	
2014	Riener et al.; 24,794,000	Colon	1068	Down	Yes. Better.	
2018	Kamal et al.; 30,140,383	Endo-metrium	163	Up	Yes. Better.	↑ AGR2 in low grade, but not high-grade endometrial cancers
2015	Alavi et al; 26,445,321	Lung	400	Up (98.3% positivity)	Yes. Poorer.	Pronounced in young patients.

outperform serum PSA monitoring when detected in urinary exosomes [47]. In cholangiocarcinoma, the same AGR2 variant (now referred to as AGR2vH) was associated with a more aggressive phenotype [46, 48]. Although AGR2vH mRNA is overexpressed in PCa and cholangiocarcinomas, the existence of specific AGR2vH translation products/proteins remains controversial, highlighting the need for splice variant-specific antibodies for future studies [47, 49].

There is experimental evidence that a functional AR is required for androgen- and estrogen-dependent regulation of AGR2 in PCa cell lines [6]. Since both PSA and AGR2 are AR-regulated proteins, one might expect a correlation between AGR2 levels and PSA serum levels. However, the present study confirms previous findings that there is no significant association between AGR2 expression in tumours and serum PSA [29].

In summary, we found AGR2 expression significantly elevated in 93.5% of primary prostate cancer tissues. A gradual loss of AGR2 expression in tumour samples (protein/mRNA) was associated with increasing tumour grade (ISUP) and shortened patient survival. No associations of AGR2 expression with other clinical–pathological parameters like PSA were found in this large-scale study. We conclude that AGR2 is not suitable as an independent prognostic marker in prostate cancer but deserves further study as a target of therapy or a serum marker.

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CONFLICT OF INTEREST

The authors have nothing to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. AGR2 immunohistochemistry evaluation scheme. (A) negative for AGR2. (B) Weak (1+) AGR2 positivity. (C) Moderate (2+) AGR2 positivity. (D) strong (3+) AGR2 expression.