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A high cannabinoid CB_1 receptor immunoreactivity is associated with disease severity and outcome in prostate cancer

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ARTICLE INFO

Article history:
Received 20 August 2008
Received in revised form
3 October 2008
Accepted 15 October 2008
Available online 4 December 2008

Keywords:
Prostate cancer
Cannabinoid receptor
Gleason score
Kaplan–Meier analysis

ABSTRACT

In the light of findings indicating that cannabinoids can affect the proliferation of a number of cancer cell types and that cannabinoid receptor expression is higher in prostate cancer cell lines than in non-malignant cells, we investigated whether the level of cannabinoid 1 receptor immunoreactivity (CB_1IR) in prostate cancer tissues is associated with disease severity and outcome. Formalin-fixed paraffin-embedded non-malignant and tumour tissue samples from patients who were diagnosed with prostate cancer at a transurethral resection for voiding problems were used. CB_1IR, which was scored in a total of 399 cases, was associated with the epithelial cell membranes, with little staining in the stroma. Patients with a tumour CB_1IR score greater or equal to the median (2) had a significantly higher proportion of Gleason scores 8–10, metastases at diagnosis, tumour size and rate of cell proliferation at diagnosis than patients with a score < 2. For 269 cases, tumour CB_1IR was measured for patients who only received palliative therapy at the end stages of the disease, allowing the influence of CB_1IR upon the disease outcome to be determined. Receiver operating characteristic (ROC) curves showed an area under the curve of 0.67 (95% confidence limits 0.59–0.74) for CB_1IR in the tumour. CB_1IR in non-malignant tissue was not associated with disease outcome. A tumour CB_1IR score ≥ 2 was associated with a significantly lower disease specific survival. A Cox proportional hazards regression indicated that the tumour CB_1IR score and the Gleason score were independent prognostic variables. It is concluded that a high tumour CB_1IR score is associated with prostate cancer severity and outcome.

1. Introduction

Prostate cancer is the major cancer form afflicting males. In the United States of America (USA), for example, the American Cancer Society listed 218,219 new cancer cases in their estimates for 2007, a number approximately equal to the number of male cases for lung and bronchus, colon and rectum, and melanoma of the skin put together.[1] There is a

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0959-8049/$ - see front matter
doi:10.1016/j.ejca.2008.10.010

wide range of treatment options for prostate cancer depending on tumour characteristics, patient age and status. However, the curative treatments for localised prostate cancer (prostatectomy and radiation therapy) are associated with the risks of side-effects, including erectile dysfunction and incontinence,[2–4] and both novel treatment strategies and prognostic markers (to avoid the overtreatment of patients who would better have been served by surveillance alone[5]) are much needed.

Δ^9-Tetrahydrocannabinol (Δ^9-THC), the main psychoactive ingredient of cannabis, produces most of its effects via the activation of two G-protein coupled cannabinoid (CB) receptors termed CB_1 and CB_2. CB_1 receptors are among the most common of all receptor types in the brain, and are also located peripherally in both neurons and non-neuronal tissue, whilst CB_2 receptors are mainly found in immune cells.[6] The endogenous ligands for these receptors ('endocannabinoids') anandamide and 2-arachidonoylglycerol are synthesised on demand and mimic many of the actions of Δ^9-THC, but have short-lived effects due to effective metabolic pathways.[7,8]

Synthetic Δ^9-THC (Marinol™) and its analogue nabilone (Cesamet™) are licensed in the USA, Canada and the United Kingdom (UK) for their palliative effects upon chemotherapy-induced nausea and vomiting. However, cannabinoids also affect the viability, proliferation, adhesion and migration of cancer cells, including prostate cancer cells, and can reduce angiogenesis and the growth of tumour cells implanted into nude mice.[9–18] In 2005, Sarfaraz and colleagues[13] reported that CB_1 receptor expression by the human prostate cancer cell lines, LNCaP (androgen-sensitive), DU145 and PC3 (androgen-independent), was higher than that seen in normal human prostate epithelial cells. Furthermore, the CB_1 receptor expression was higher in CA-HPAV10 cells (virally transformed cells from the adenocarcinoma of human prostate tissue) than in the corresponding CA-HPV-7 cells, also virally transformed, but from normal human tissue.[13] It is not known, however, whether the level of CB_1 receptor immunoreactivity (CB_1IR) is associated with disease severity and outcome in prostate cancer tissue samples from patients. This question has been addressed in the present study.

2. Materials and methods

2.1. Patients

The formalin-fixed, paraffin-embedded samples used in the present study were from a large series of cases diagnosed with prostate cancer at transurethral resection for micturation difficulties, as reported in detail previously.[19] The samples were collected at the Central Hospital, Västerås, Sweden, between 1975 and 1991, i.e. before serum prostate specific antigen (PSA) was used in Sweden as a diagnostic tool. Evidence for metastases was undertaken using a bone scan shortly after the transurethral resection, and the patients were followed up until 2003. For the 399 cases where either tumour and/or non-malignant tissue CB_1IR could be scored (see below), 297 were followed up by watchful waiting until the appearance of metastases (the standard treatment approach at the time) and the others received hormonal treatment, radiotherapy or radical prostatectomy. Cause of death was assessed by the evaluation of medical records. The Gleason scores and the percentage of the specimen area that was tumour-associated (%Ca) were assessed in each sample by one pathologist (LE). To facilitate screening for markers related to outcome, tissue micro arrays were constructed using a Beecher Instrument (Sun Prairie, WI, USA). The tissue micro arrays contained up to eight (usually five) samples of tumour tissue (cores with a diameter of 0.6 mm) and up to four samples of non-malignant tissue from each patient.[20,21] Care was taken to ensure that tumour cores from both primary and secondary Gleason score areas were included in the micro arrays.

2.2. Immunohistochemistry

The paraffin-embedded tissue micro array sections were deparaffinised and rehydrated. Subsequently, sections were placed in a citrate buffer pH 6.0 and boiled in a pressure cooker for 60 min. Samples were then placed in water and then in Ventana buffer, after which they were placed in a Ventana automated analyser (Ventana Medical Systems Inc., Tucson, AZ) to which the CB_1 receptor antibody (AbCam cat. no. 23703, lot no. 280229, dilution 1:300; AbCam plc, Cambridge, UK) and the secondary system (iVIEW DAB Detection Kit, Ventana Medical Systems Inc.) were added. Mouse forebrain and prostate tissues were also investigated in the same manner using both lot nos. 280229 and 396756 of the antibody, a rabbit polyclonal raised to a peptide corresponding to C terminal amino acids 461–472 of the human CB_1 receptor which cross-reacts with the human, mouse and rat CB_1 receptor according to the data obtained from the manufacturers. Ki-67 scores, determined by assessing the % of cells (500 cells per patient, evaluated in 5–7 areas per tumour) expressing Ki-67 immunoreactivity (antibody MIB-1, DAKO Corporation, Carpenteria, CA, USA), were available in our database (for a previous study see[22]) undertaken in some of the present cases. The scores ranged from 0% to 78.5%, and the division of data here as <2 and >2 represents a simple division around the median.

2.3. Scoring of CB_1IR

The individual cores were scored for CB_1IR by an evaluator (SC) who was blind to the clinical data for the patients. Each core was scored for immunoreactive intensity (0–3 where 0 is absent and 3 is high) and epithelial cell distribution (0%, 10%, 33%, 50% and 100%) for each intensity to give a composite value between 0 and 3. A case, for example, with 50% intensity 2 and 50% intensity 3 in the epithelial cells would receive a composite score for that core of 2.5. A case with 10% intensity 3 and equal intensities 1 and 2 would receive a composite score of $3 \times 0.1 + [(1 + 2)/2] \times 0.9 = 1.65$. The median score for the cores was then calculated for each patient. Usually, 5 (and in some cases 8) tumour and 4 non-malignant tissue cores per patient were analysed for CB_1IR, the main exceptions being when the core consisted of stroma alone, or the quality of the core was not sufficiently good for immunoreactivity to be scored with confidence. Cases where the structure or quality of the tissue and/or staining was unclear were discussed with an expert pathologist (AB) prior to decid-

ing the score and/or inclusion in the dataset. From a total of 2691 scored cores, median scores from 372 (tumour tissue) and 349 (non-malignant tissue) patients were determined.

2.4. Statistics

Receiver operating characteristic (ROC) curves were used to define cut-offs for CB_1IR. In survival analyses with Kaplan–Meier or Cox's regression an event was defined as prostate cancer death. Death from other causes was censored, and patients alive were censored at the date of last follow-up. The duration of event-free survival (EFS) is defined as the time from diagnosis until the date of prostate cancer death, death of other causes or if no death occurred, until the date of last follow-up. Survival curves were constructed using the Kaplan–Meier method, and the differences in outcome between groups were tested with the log-rank test. The prognostic relevance of CB_1IR was examined by Cox's regression analysis alone and together with the known prognostic factor Gleason score, as covariable. Differences in the distribution of variables between the groups of patients were analysed using the chi-square test. The level of statistical significance was defined as $p < 0.05$ (two-sided). Statistical analyses were conducted using SPSS 14.0.0 software for Windows (SPSS Inc., Chicago, IL, USA) and the statistical package built into the GraphPad Prism computer programme (GraphPad Software Inc., San Diego, CA, USA).

3. Results

3.1. Antibody characterisation in brain tissue

A recent study has indicated that the specificity of a number of commercially available CB_1 receptor antibodies can be an issue.[23] The antibody used in our investigation (AbCam antibody no. 23703, lot no. 280229) was not included in that study, but in a preliminary study we found that it produced the appropriate pattern of staining in paraffin-embedded, formalin-fixed sections from human cerebellum, where the CB_1 receptor distribution is known (data not shown). The specificity of the antibody was also verified by lack of staining in mice forebrain tissue from animals where the CB_1 was knocked-out, in contrast to the extensive staining in wild-type mice (Appendix 1A and B). Formalin-fixed prostate tissues from wild-type, heterozygote and $CB_1^{-/-}$ mice were also investigated, and a reasonable degree of specificity could be observed in view of the relatively low intensities of staining in the $CB_1^{+/+}$ and $CB_1^{+/-}$ tissues (Appendix 1C). A later batch of the antibody (lot. no. 396756) was also tested. The pattern of forebrain staining was the same as seen with lot no. 280229 (i.e. $CB_1^{+/+}$ and $CB_1^{+/-} > CB_1^{-/-}$) but the staining intensity of the wild-type and heterozygote prostate tissue was lower with lot no. 396756 than with lot no. 280229 (data not shown).

3.2. CB_1 receptor immunoreactivity (CB_1IR) in non-malignant prostate tissue

CB_1IR was assessed, using lot no. 280229 of the antibody, in replicate samples of non-malignant tissue taken at transurethral resection from 349 patients. The staining intensity varied between samples, but CB_1IR was localised to the plasma membrane of both basal and luminal epithelial cells, and with little or no staining of the stroma. Some of the epithelial cells in the basal and luminal compartment were more intensely stained than others. The reason for this heterogeneity was not further explored, other than to note that the relative number of intensely stained cells was higher than would be expected if the high CB_1IR was due to selective expression on neuroendocrine cells. An example from a core with a strong CB_1IR is shown in Fig. 1.

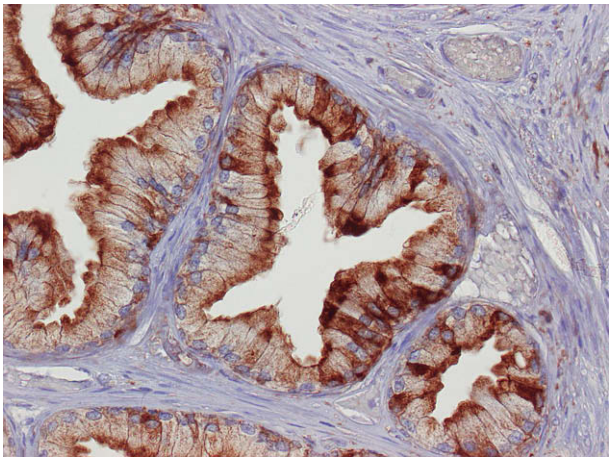

Fig. 1 – CB_1IR in a core of non-malignant tissue obtained at transurethral resection from a 76-year-old patient. The magnification used was 40×.

3.3. Comparison of CB_1IR in tumour and normal tissue

A total of 372 tumour cases could be assessed, and representative examples are shown in Fig. 2. As for the normal tissue, the CB_1IR in the well-differentiated tumours was associated with the epithelial cells rather than with the stroma (Fig. 2A and B). In some cases, a weak staining was seen for blood cells found in the core (see e.g. Fig. 2C, top right). This is consistent with the reports that B- and T-cells express mRNA for CB_1 receptors, particularly after activation.[24,25] Examples of cores scored as 1, 2 and 3 are shown in Fig. 2B, and an example of a core showing all three scores within the same sample is shown in Fig. 2A.

Median scores from replicate cores of non-malignant and tumour tissue samples were calculated as described in Section 2. Examples of two tumour tissue series are shown in Fig. 2C. The distribution of the median values for the 372 tumour and 349 non-malignant tissue samples is shown in Appendix 2. In both instances, the group median score was 2 and indeed, this score was very common, representing 31% and 29% of the median scores for the tumour and non-malignant tissue samples, respectively. Comparison of the absolute values for the 322 samples, where data for both non-malignant and tumour tissue were available indicated that the CB_1IR scores for the tumour and non-malignant samples were significantly different ($p < 0.001$, Wilcoxon signed ranks test). On the basis of receiver operating characteristic

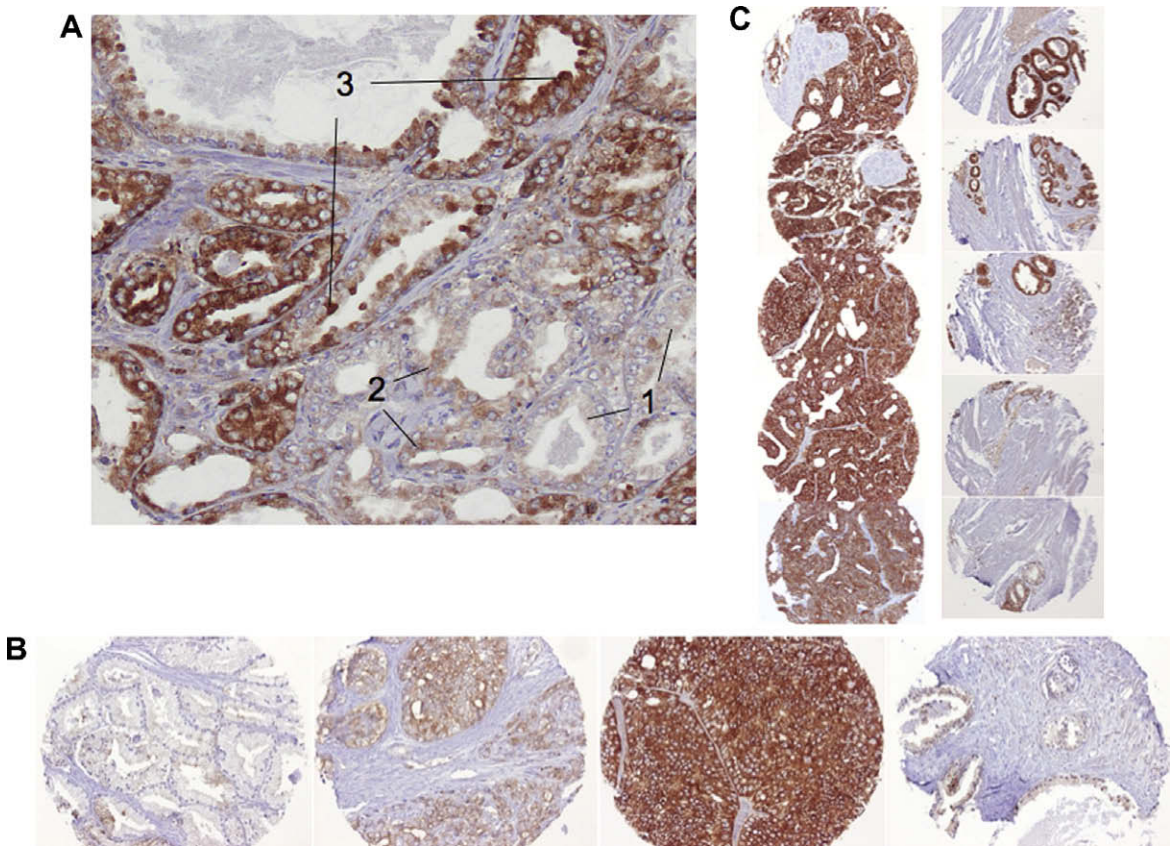

Fig. 2 – CB_1IR in prostate cancer tumour tissue obtained at transurethral resection. Panel A: tissue from a 74-year-old patient (magnification 20×). The tumour size was 10% of the resected tissue, and was given a Gleason score of 6. The numbers in the figure indicate the CB_1IR scores for the individual cells. Panel B: left three images: tumour tissue cores with CB_1IR scores of 1, 2 and 3, respectively. The far right image is a non-malignant tissue core from the same patient who scored 3. Panel C: left column, replicate cores of tumour tissue from a 69-year-old patient, tumour size 95%, Gleason score 8. All five cores were given a CB_1IR score of 3. In the right column, replicate cores from a 71-year-old patient (tumour size 25%, Gleason score 8) are shown. The CB_1IR scores ranged from 3 (top) to 1.5 (bottom), giving a median value of 2. Note that the brightness of the images was adjusted digitally to give similar levels of the background, and that the bottom core in panel B left column was photographed on a different occasion to the others.

(ROC) curves described below, the CB_1IR scores were split into two groups, <2 and ⩾2, for further analysis.

The clinical data for the patients with CB_1IR scores < 2 and ⩾2 are shown in Table 1. For the tumour samples, 42% of the patients in the ⩾2 group had Gleason scores of 8–10, as compared with 12% in the <2 group. The incidence of metastases at diagnosis was also higher in the ⩾2 group (17%) than in the <2 group (5%). Since a previous study has shown that a value of >20 for the percent of the specimen that is cancer associated (%Ca) is indicative of a poor prognosis,[22] this parameter was also investigated. The number of patients with %Ca > 20 was higher in the ⩾2 group (61%) than in the <2 group (38%), suggesting that a high CB_1IR may be associated with cell proliferation. These differences were not seen in the non-malignant tissue samples, although there was a variation in the distribution of Gleason scores (Table 1). Data for a marker for cell proliferation, Ki-67 immunoreactivity, were available for a subset of the patients, and the number of patients with a tumour Ki-67 score above its median value of 2 was higher in the CB_1IR ⩾ 2 group (58%) than in the <2 group (15%) (Table 1). A total of 30 patients with Gleason scores of 6–7 were scored for both tumour Ki-67 and CB_1IR. The small sample size precludes statistically meaningful interpretation of the data, but the percentages 14% (1/7 cases) and 39% (9/23 cases) showing Ki-67 scores > 2 for the CB_1IR < 2 and ⩾2 groups, respectively, are at least consistent with the whole data set.

3.4. *Disease-specific survival in patient groups with CB_1IR scores < 2 and ⩾2*

During the period when the samples were obtained, the standard treatment in Sweden was watchful waiting until the appearance of metastases. In all, a total of 269 of the 372 cases where tumour CB_1IR was assessed (and 275 of the 349 cases for non-malignant tissue) did not receive any curative treatment for their cancer, thereby allowing the evaluation of factors such as the expression level of a given protein upon the

Table 1 – Characteristics of the tumour and non-malignant tissue samples obtained at transurethral resection with cannabinoid 1 receptor immunoreactivity (CB_1IR) scores < 2 or ≥2.

		$CB_1IR < 2$	$CB_1IR \geq 2$	p-Value[a]
Tumour tissue samples		*n* = 92	*n* = 280	
Age (mean ± SD) at diagnosis		73.6 ± 6.6	73.7 ± 7.5	NS
Age range at diagnosis		58.3–91.8	51.0–95.0	
Sampling period		1975–1991	1975–1991	
Gleason score at diagnosis	4–5	26 (28%)	58 (21%)	<0.001
	6	41 (45%)	56 (20%)	
	7	14 (15%)	49 (18%)	
	8–10	11 (12%)	117 (42%)	
Metastases at diagnosis[b]	No	70 (95%)	180 (83%)	<0.05
	Yes	4 (5%)	38 (17%)	
%Ca	≤20%	57 (62%)	110 (39%)	<0.001
	>20%	35 (38%)	170 (61%)	
Median Ki-67 score[c]	<2	17 (85%)	35 (42%)	<0.001
	>2	3 (15%)	49 (58%)	
Non-malignant tissue samples		*n* = 134	*n* = 215	
Age (mean ± SD) at diagnosis		75.1 ± 7.0	73.4 ± 7.2	<0.05
Age range at diagnosis		56.1–95.0	53.0–91.8	
Sampling period		1975–1991	1976–1991	
Gleason score (for the corresponding tumour tissue) at diagnosis	4–5	29 (21.6%)	64 (29.8%)	<0.01
	6	51 (38.1%)	49 (22.8%)	
	7	22 (16.4%)	30 (14.0%)	
	8–10	32 (23.9%)	72 (33.5%)	
Metastases at diagnosis[c]	No	90 (90.0%)	147 (88.0%)	NS
	Yes	10 (10.0%)	20 (12.0%)	
%Ca	≤20%	79 (59.0%)	104 (48.4%)	NS
	>20%	55 (41.0%)	111 (51.6%)	

a p-Values are determined using either chi-square tests or (in the case of the ages at diagnosis) two-tailed unpaired t-tests. NS: not significant.
b In 80 (tumour) and 82 (normal) cases, the metastatic state at diagnosis was not known.
c Data for the 104 cases where both tumour tissue Ki-67 and CB_1IR scores were obtained. There was no significant correlation between the tumour Ki-67 and normal tissue CB_1IR scores (n = 85).

natural progression of the disease. The median age of the 269 cases at diagnosis was 74.5 years.

Receiver operating characteristic (ROC) curves using a 15-year cut-off showed an area under the curve of 0.67 (95% confidence limits 0.59–0.74, $p < 0.001$) for CB_1IR in the tumour and a division of the data into <2 and ⩾2 was the most appropriate (data not shown). In contrast, the ROC curve for the non-malignant tissue gave an area under the curve that was not significantly different from 0.5, indicating that it was not significantly associated with disease outcome (data not shown). ROC curves using a 15-year cut-off were also constructed for the tumour samples, where the highest score for an individual core from each patient (rather than the median value of the cores) was used. As with the median values for the tumour samples, the area under the curve was statistically significant (0.64, 95% confidence interval 0.56–0.72, $p < 0.001$).

Kaplan–Meier analysis of data for the 269 patients followed up with watchful waiting showed that patients with median CB_1IR scores ⩾ 2 had poorer outcomes than patients with $CB_1IR < 2$ (Fig. 3A). The hazard ratio (Mantel-Haenszel, prism 5) was 2.46, with 95% confidence limits of 1.48–4.08 (i.e. $p < 0.05$). When the cases were randomly split into two groups of n = 135 and 134, the significant contribution of the CB_1IR scores was still seen in both groups (data not shown). As expected from the ROC analysis, the CB_1IR score for the non-metastatic tissue samples did not contribute significantly to the fraction survival (Appendix 3). For the 136 men below median age, the association of tumour CB_1IR expression with disease outcome remained significant ($p < 0.005$), but this was not the case for the 133 patients above the median age ($p = 0.08$) (data not shown). As pointed out above, %Ca is a good prognostic marker.[22] There was no significant contribution of the CB_1IR score in the 146 patients with %Ca ⩽ 20 ($p = 0.2$) (data not shown). However, the patients with a CB_1IR ⩾ 2 and a %Ca > 20 showed a significantly worse survival rate than those with a $CB_1IR < 2$ and a %Ca > 20 (hazard ratio 2.51, with 95% confidence limits of 1.43–4.43. $p < 0.05$) (Fig. 3B).

3.5. *Disease-specific survival in patient groups with CB_1IR scores < 2 and ⩾ 2 – relation to Gleason score*

A Cox proportional hazards regression analysis indicated that the CB_1IR score and the Gleason score were independent variables associated with disease outcome (Table 2). The size of the patient material allows Kaplan–Meier curves to be drawn

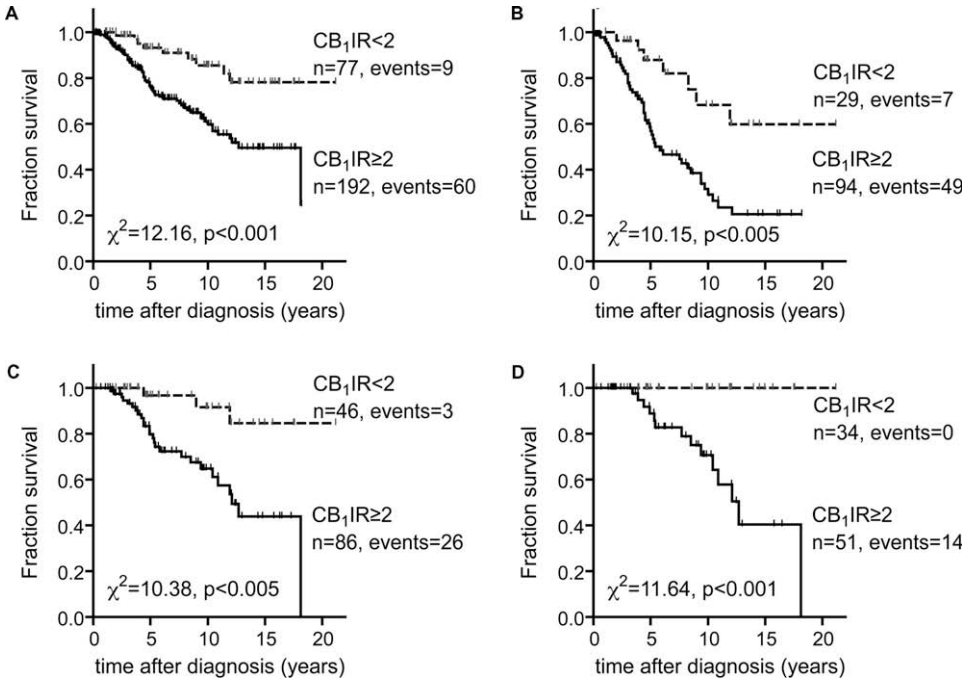

Fig. 3 – Kaplan–Meier plots for the fraction survival of patients followed with active expectancy with tumour CB_1IR scores of <2 or ≥2. Panel A: all patients (*n* = 269); B: patients with %Ca scores > 20; C: patients with Gleason scores 6 or 7; and D: patients with Gleason scores of 6. The hatches on the lines indicate the censored data (i.e. cases other than death due to prostate cancer). The 15-year probabilities of event-free survival for CB_1IR scores of <2 or ≥2, respectively, were A: 78 ± 7% compared to 50 ± 5%; B: 60 ± 13% compared to 21 ± 6%; C: 85 ± 9% compared to 44 ± 9%; and D: 100% compared to 40 ± 13%.

for subsets with Gleason scores of 6–7 and 6 alone. In both subsets, there was a significant association of the CB_1IR scores with disease outcome (Fig. 3C and D). The hazard ratios could not be defined for the Gleason score 6 subset since there were no events in the $CB_1IR < 2$ group, but for the Gleason score 6–7 subset, the value was 3.44 (95% confidence limits 1.62–7.29, i.e. $p < 0.05$).

Table 2 – Cox's regression for tumour CB_1IR.

Variable		*n*	RR	*p*-Value	95% CI
(A) Univariate analysis					
GS	4–5	91	1[a]		
	6–7	150	25.0	0.002	3.4–182.9
	8–10	63	128.7	<0.001	17.6–939.5
Tumour CB_1IR	<2	77	1[a]		
	≥2	192	3.2	0.001	1.6–6.5
(B) Multivariate analysis					
GS	4–5	78	1[a]		
	6–7	132	24.6	0.002	3.4–181.1
	8–10	59	130.9	<0.001	17.8–961.8
Tumour CB_1IR	<2	77	1[a]		
	≥2	192	2.7	0.006	1.3–5.5

Abbreviations: RR, relative risk; CI, confidence interval; and GS, Gleason score.
a Reference value.

4. Discussion

The present study was motivated by the studies done on cultured prostate cancer cells indicating that the levels of CB_1 receptors are higher than those in non-malignant cells,[13] and that cannabinoids affect the viability and/or invasivity of such cells.[13–18] We have found that the level of CB_1IR in tumour tissue, but not in non-malignant tissue, as determined by using the AbCam antibody no. 23703 (lot no. 280229) is associated with disease severity and outcome. The presence of CB_1IR in the human prostate confirms the data of Galiègue and colleagues[26] and Ruiz-Llorente and colleagues[27] who found detectable levels of mRNA for CB_1, but not for CB_2 receptors, in human prostate tissue. CB_1IR was associated with the epithelial cells and not with the stroma of both non-malignant tissue and differentiated tumour tissue. This is consistent with a recent study reporting CB_1IR (but not CB_2IR) in the epithelial cells, but not in the stroma, of the rat prostate, where they are involved in the modulation of contraction of this tissue,[28] and with the human prostate, where they show the appropriate pertussis toxin-sensitive inhibitory coupling to adenylyl cyclase.[27] Albeit with a low (and variable) staining intensity, an epithelial localisation of CB_1IR is also seen in the mouse prostate (Appendix 1C).

For the 372 tumour cases, but not for the 349 non-malignant tissue cases, there was a higher proportion of Gleason score 8–10 and metastasis (at diagnosis) for the $CB_1IR \geqslant 2$ group than the <2 group, suggesting that the immunoreactive score is related to disease severity at diagnosis. In addition,

there was a clear association between CB_1IR and tumour size in the specimen taken at transurethral resection. Although there does not appear to be an endocannabinoid tone in the normal rat prostate,[27] cultured human WPMY-1 prostate stromal cells release 2-arachidonoylglycerol[29] suggesting a potential stromal influence on CB_1 receptors in prostate cancer. CB_1 receptors are coupled to a variety of signalling cascades, including the inhibition of cyclic AMP and activation of the extracellular signal-related kinase pathway.[6] The extracellular signal-related kinase pathway can regulate both cell proliferation and cell death,[30] raising the possibility that tumour CB_1 expression, by increasing extracellular signal-related kinase signalling in the appropriate manner in response to locally released endocannabinoids, could contribute to prostate cancer cell growth. The correlation between the tumour CB_1IR and Ki-67 scores in the present study would support the hypothesis. This is analogous to the situation in the mouse skin, where animals lacking CB receptors have attenuated mitogen activated protein kinase and NFκB responses, as well as a decreased incidence of skin carcinogenesis in response to ultraviolet UVB irradiation following 7,12-dimethyl benz(a)anthracene initiation as compared to wild-type mice.[31] This is somewhat at odds with the data suggesting that cannabinoids can affect cell proliferation and cell invasion,[13–15,17,18] but these data were undertaken using cell lines, and there has been a report of a pro-cancer action of a CB receptor agonist in a prostate cancer cell line,[16] so the picture is far from clear.

An alternative possibility that is more in line with the cell line data is that the expression of CB_1IR is regulated by the local endocannabinoid release. In this scenario, a low endocannabinoid tone, which would allow for an increased rate of proliferation, results in a compensatory increase in surface expression of CB_1 receptors. In a recent study, it was found that the invasivity of prostate cancer cell lines is dependent upon its activity of fatty acid amide hydrolase (FAAH) the enzyme responsible for metabolism of the endocannabinoid anandamide.[32] These authors also reported that the expression levels of FAAH were considerably higher in prostate cancer tissues than in normal prostate tissues.[32] Although the level of expression was not associated with tumour differentiation (Gleason score),[32] the results motivate an investigation of endocannabinoid synthetic and degradative enzymes in our patient series, in order to see whether the expression of CB_1IR is correlated with such markers.

The use of a large sample size of well-characterised, untreated patients with a long follow-up period has allowed for the determination of the association of CB_1IR with disease outcome. A significant association was seen in the tumour tissue, but not for the non-malignant tissue, suggesting that it reflects a local change rather than a general underlying vulnerability that would be seen, for example, in blood samples. To our knowledge, there are only two studies investigating the association of CB_1 receptor expression with disease outcome in cancer in general, and none for prostate cancer. Michalski and colleagues[33] recently reported data from 37 pancreatic tumour samples. A high CB_1IR was associated with a shorter survival time (median 6 months) than a low CB_1IR (median 16 months). These data were corroborated by the authors with quantitative RT-PCR in a separate cohort of 53 cancer samples.[33] In contrast, disease-free survival in hepatocellular carcinoma was reported to be lower in 35 patients with a low CB_1IR than in the 29 patients with a high CB_1IR[34] suggesting that the role of the CB_1 receptor is highly dependent upon the cancer form studied.

In conclusion, the present study has extended the studies using cultured cells[13] to demonstrate that a high CB_1IR immunoreactivity is associated with a more severe form of the disease at diagnosis and a poorer outcome. Future studies investigating other components of the endocannabinoid system in our patient series are clearly warranted, as are investigations of the CB_1IR in metastatic tissue. The finding that the CB_1IR is associated with disease outcome in the patients with Gleason scores of 6–7 and 6 alone is of potential clinical importance, given that treatment decisions are difficult for such patients.

Conflict of interest statement

None declared.

Acknowledgements

The authors thank Pernilla Andersson and Eva Hallin for their expert technical assistance. The authors are indebted to Dr. Thomas Brännström for providing us with the human cerebellum sample, and Drs. Michelle Glass, Scott Graham, Michael Elphick and Thomas Brännström for their help and advice concerning the assessment of CB_1 receptor immunoreactivity in this sample. We are grateful to Dr. Hans Stenlund for his advice on the statistical treatment of the data. The authors also thank the Swedish Science Council (Grant No. 12158, medicine, C.J. Fowler); the Swedish Cancer Society (Grant No. CAN 2007/712, Anders Bergh; Grant No. CAN 2007/693, Pär Stattin); and the Research Funds of the Medical Faculty, Umeå University (C.J. Fowler) for the financial support.

Appendix 1

CB_1IR in forebrain samples from mice either expressing (panel A) or lacking (panel B) receptors. Paraffin-embedded, formalin-fixed specimens were obtained from wild-type ($CB_1^{+/+}$), heterozygote ($CB_1^{+/-}$) and knock-out ($CB_1^{-/-}$) mice that were generated and genotyped as described previously.[35] The insets show a low magnification (4×) forebrain, whilst a part of the forebrain at a higher magnification (20×) is shown in the main figures. In panel C, CB_1IR for prostate tissue (magnification 20×) is shown (see Fig. A1).

Appendix 2

Frequency distribution of the CB_1IR scores for the non-malignant ($n = 372$) and tumour ($n = 349$) tissue. The data are grouped in the blocks of 0.5 around the value ('bin center') given on the abscissae. In the inset, the corresponding values are shown for the blocks of 0.1 for bins in the vicinity of the median value for the entire population (see Fig. A2).

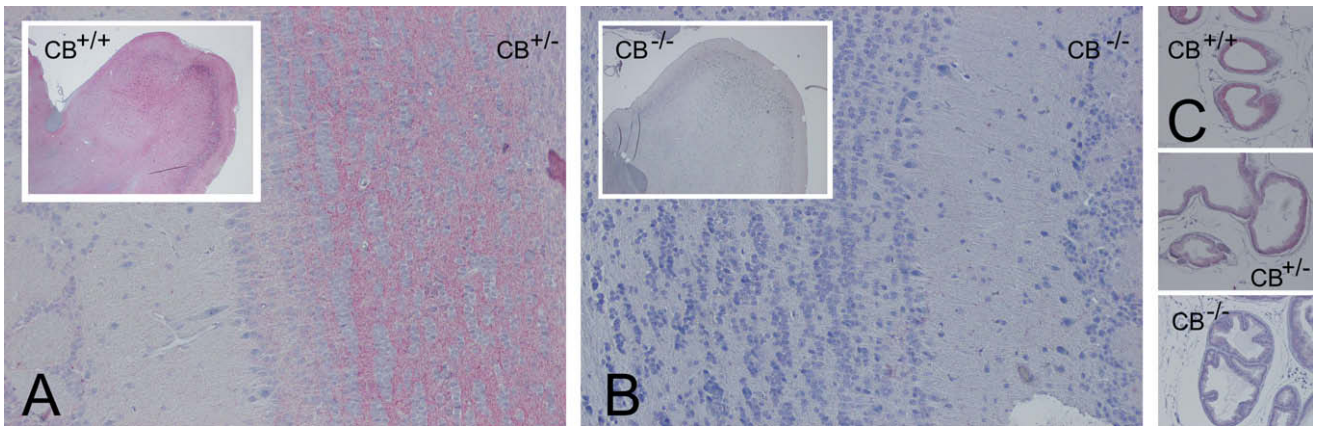

Fig. A1

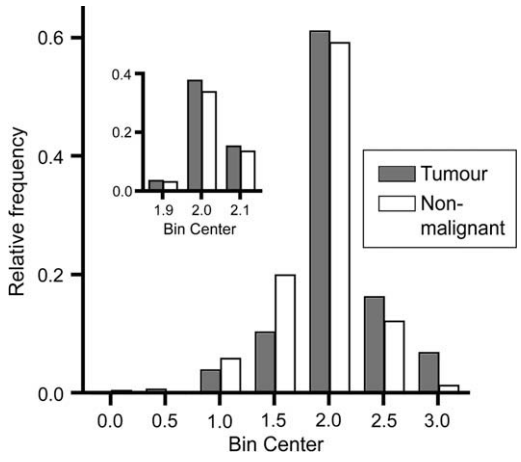

Fig. A2

Appendix 3

Kaplan–Meier plots for the fraction survival of untreated patients with non-malignant tissue CB_1IR scores of <2 or ⩾2 (see Fig. A3).

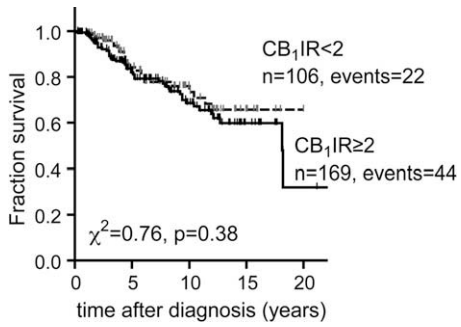

Fig. A3

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