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HER2 and COX2 expression in human prostate cancer

J. Edwards, R. Mukherjee, A.F. Munro, A.C. Wells, A. Almushatat, J.M.S. Bartlett*

Endocrine Cancer Research Group, Section of Surgical and Translational Research, Division of Cancer Sciences and Molecular Pathology, University Department of Surgery, Level II Queen Elizabeth Building, Glasgow Royal Infirmary, Glasgow, Scotland G31 2ER, UK

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Abstract

COX2 and HER2 expression are associated with a poor prognosis in prostate cancer and HER2 has been linked to COX2 expression in colorectal cancer. The association between COX2 and HER2 expression was investigated in 117 patients with prostate cancer (89) or Benign prostatic hyperplasia (BPH) (28). Tissue was analysed for *HER2* amplification by fluorescent *in situ* hybridisation, and HER2 and COX2 protein expression by immunohistochemistry (IHC). All tumours analysed expressed COX2 at a significantly higher level than BPH tissue ($P = 0.041$). Only low levels of *HER2* gene amplification (8%, 7/89) and HER2 protein expression (12%, 11/89) were observed. HER2 protein expression was rarely observed and did not correlate with *HER2* amplification or COX2 expression. Although HER2 does not drive COX2 expression in prostate cancer, this study identified high levels of COX2 expressed in locally advanced prostate cancer, suggesting COX2 could be a potential therapeutic target. COX2 inhibitors are currently being used in clinical trials for the treatment of other tumour types.

Keywords: Gene amplification; Protein expression

1. Introduction

There are two isoforms of the enzyme which catalyses the conversion of arachidonic acid to prostanoids, the constitutively expressed cyclooxygenase 1 (COX1) and the inducible isoform cyclooxygenase 2 (COX2) [1,2]. COX2 expression is increased in response to pro-inflammatory stimuli [3], tumour promoters [4] and growth factors [5]. COX2 is expressed in many solid tumour types including colorectal, breast, prostate and ovarian tumours [6–10].

In some human cancers, a link between COX2 and HER2, a type one tyrosine kinase receptor, has been described [5–7,11]. In colorectal cancer, activation of HER2 induces activation of COX2 promoters [6]. COX2 expression may also be regulated by activation of the mitogen activated protein (MAP) kinase cascade via HER2 in breast carcinoma cells *in vitro* [7].

Increased COX2 expression has been associated with high prostate tumour grade [12]. COX2 is proposed to influence prostate cancer progression and development via neoangiogenesis (the formation of new blood vessels) and increased resistance to apoptosis [13–15]. Prostate cancer cells expressing COX2 secrete the prostanoid PGE2, which stimulates production of the angiogenic vascular endothelial growth factor (VEGF) [13]. *In vivo* COX2 inhibitors block VEGF production and decrease tumour microvessel density in prostate cancer [13,14]. Increased COX2 expression in prostate cancer cells *in vitro* correlates with a decrease in the apoptotic index and COX 2 inhibitors induce apoptosis [15,16]. COX2 therefore appears to have a role in cancer of the prostate. However, no one to our knowledge has as yet explored the link between COX2 and HER2 expression in prostate cancer.

The *HER2* oncogene is amplified in 25–30% of breast and ovarian tumours [17,18] and is associated with a poor prognosis [19]. *HER2* is the second member of the epidermal growth factor receptor (EGFR) tyrosine kinase growth factor receptor family [20] which are

* Corresponding author. Tel.: +44-141-211-5436; fax: +44-141-211-5432.

E-mail address: j.m.bartlett@clinmed.gla.ac.uk (J.M.S. Bartlett).

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important mediators of cell growth, differentiation and survival [21,22].

The frequency and significance of *HER2* amplification in prostate cancer remains to be established. Reports of frequency of HER2 overexpression vary widely as published studies use a variety of antibodies with differing sensitivities and specificity's [21,23–27]. HER2 overexpression has been correlated with a high Gleason score, stage and poor prognosis [27–29] and also shown to have no correlation to Gleason score, stage and prognosis [24,25]. *HER2* gene amplification rates reported in prostate cancer range from 41 to 0% [20,30,31] and has recently been linked to the development of androgen escape [21,32].

This study investigated the role of HER2 and COX2 in 117 prostate samples from a patient cohort of Benign prostatic hyperplasia (BPH), pT1, pT2, pT3 and pT4 tumours at diagnosis. By using validated methods to assess HER2 status [18], we aimed to clarify the controversy surrounding HER2 status in prostate cancer.

2. Patients and methods

117 patients were retrospectively selected for analysis, ethical approval was obtained from the local research and ethical committee for use of tissue in this study. Patients selected for analysis included: those with BPH [28], those with stage pT1 or pT2 prostate cancer at diagnosis (53) and those with pT3 or pT4 prostate cancer at diagnosis [37]. Prostate-specific antigen (PSA) values and Gleason scores were available for each patient with prostate cancer.

2.1. Immunohistochemistry

2.1.1. COX2

Tissues sections (5 μM) were dewaxed in xylene and rehydrated through graded alcohols. Antigen retrieval was carried out by heating in Tris–ethylene diamine tetraacetic acid (EDTA) buffer (pH8) for 5 min under pressure (15 p.s.i.) in a microwave. The tissue was stained with a COX2 (human) monoclonal antibody (Cayman Chemicals, USA) at a concentration of 0.625 mg/ml and a biotinylated secondary antibody, streptavidin peroxidase complex and enhanced 3,3′-diaminobenzidine (DAB) as a substrate. Finally, the tissues were counter-stained with haematoxylin and dehydrated through graded alcohols and xylene. Negative control sections were incubated with an iso-type matched control antibody at a concentration of 1 mg/ml.

2.1.2. HER2

HER2 immunohistochemistry (IHC) was performed using the DAKO HercepTest™, with strict adherence to the kit protocol. Antigen retrieval was carried out in a heated water bath in Epitope retrieval solution provided for 40 min at 95–99 °C. Sections were stained using rabbit anti-human HER2 polyclonal antibody (7.5 g/ml, 0.05 M) and staining developed with the kit visualisation reagent and DAB.

Staining was scored blind by two independent observers using a weighted histoscore method [33]. Histoscores were calculated from the sum of (1×% cells staining weakly positive) + (2×% cell staining moderately positive) + (3×% cells staining strongly positive) with a maximum of 300. The mean of the two observers' scores were used for the analysis.

2.1.3. *Fluorescent* in situ *hybridization (FISH)*

Sections (5 μm) cut from archival formalin-fixed, paraffin-embedded tissue were placed on aminopropyltriethoxysilane (silane)-treated slides. The slides were pre-treated on a VP2000 robotic slide processor (Vysis, UK Ltd). This involved dewaxing and rehydration, treatment with 8% sodium thiosulphate at 80 °C for 30 min and digestion with 0.05% pepsin at 37 °C for 26 min. Tissue sections were subsequently fixed in 10% formalin for 10 min and dehydrated through increasing concentrations of ethanol.

DNA within the tissue was then denatured in denaturing solution (70% formamide, 2×SSC, pH 7.5) at 72 °C for 5 min on an Omnislide *in situ* PCR block (Thermo Hybaid, UK), then dehydrated in ethanol. The tissue was then hybridised at 37 °C on an Omnislide *in situ* PCR block (Thermo Hybaid, UK) with a probe from the Pathvysion™ kit (Vysis, UK Ltd). This incorporated a Spectrum Green™ labelled alpha satellite probe for chromosome 17 and a Spectrum Orange™ labelled *HER2* probe. Slides were then washed in 0.4×SSC, 0.3% Nonidet P-40 (NP-40) at 72 °C for 2 min, allowed to air dry and mounted in 0.5 μg/ml 4,6-diamindino-2 phenylindole-2 hydrochloride (DAPI) in Vectashield antifade (Vetrolabs, UK). Signals were visualised using a DMLB Microscope 100 W mercury lamp (Leica, UK) with a triple band pass filter block specific for the excitation and emission wavelengths of the Spectrum Green™ and Spectrum Orange™ fluors (Vysis, UK, Ltd).

Serially-sectioned haematoxylin and eosin-stained tissue sections were first examined microscopically to localise the tumour areas. FISH stained sections were then scanned at ×100 magnification to localise the tumour areas. Twenty non-over lapping nuclei per section were evaluated from three different areas by two independent observers. Signals per nucleus for chromosome 17 (green) and *HER2* (orange) were counted on a cell by cell basis and the results recorded manually. The mean chromosomal copy numbers for chromosome 17 and *HER2* were calculated. Analysis of copy number in thin tissue sections can be affected by nuclear truncation

[34]. Therefore, the normal range for chromosome 17 and *HER2* copy number were identified using the mean chromosomal copy number from 10 BPH samples, ±2 standard deviations (S.D.) (95% Confidence Intervals (CI)). Using this approach, the normal range for *HER2* and chromosome 17 were defined as 1.95±0.15 (mean ±S.D. of control values) and 1.91±0.12, respectively. Tumours with more than 2.10 or 2.03 signals per nucleus were identified as having increased *HER2* or chromosome 17 copy number, respectively. The observed *HER2*:chromosome 17 ratio in 10 BPH samples was 1.02±0.09. However, in line with current United Kingdom (UK) guidelines for the assessment of *HER2* gene amplification, amplification was defined as a *HER2*:chromosome 17 ratio greater than 2 [18]. If tumours were heterogeneous, the area with the most abnormalities was used in the final analysis.

2.2. Statistical analysis

Mann–Whitney tests were used to compare the Gleason sum and patient's age. Fishers exact test were used to compare the level of chromosome 17 copy number, *HER2* copy number and *HER2* gene amplification between groups. Dunnetts *t*-test was used to compare median COX2 histoscores between groups and Spearman Ranks Correlation Coefficient was used to assess if there was a correlation between HER2 amplification or HER2 copy number and COX2 or Gleason sum.

3. Results

117 patients were retrospectively recruited for this study, 28 with BPH, 53 with stage pT1/pT2 prostate cancer at diagnosis and 36 with stage pT3/pT4 prostate cancer at diagnosis. 22 prostate cancer patients had metastases at diagnosis, (12 pT1/pT2 and 10 pT3/pT4 stage prostate cancer at diagnosis). Median age, mean Gleason sum and median survival for all patients are shown in Table 1, where patients are either split by tumour stage or by the presence of metastases at diagnosis.

3.1. COX2 expression

All samples analysed in this study expressed COX2 at some level, 59% of tumours had high COX2 expression (defined as a histoscore of above the midpoint value of 150). COX2 expression appeared to be normally distributed about a mean value of 170 with a S.D. of 60. Prostate tumours expressed significantly higher levels of COX2 than prostate tissue with BPH ($P=0.041$) (Table 2). When tumour samples were subdivided into pT1/pT2 and pT3/pT4 staged tumours at diagnosis, pT3/pT4 tumours at diagnosis were demonstrated to express significantly higher levels of COX2 compared with BPH or pT1/pT2 ($P=0.009$ and $P=0.019$, respectively). Conversely, pT1/pT2 tumours did not show significantly higher levels of COX2 expression than BPH samples ($P=0.41$). COX2 expression was not significantly different between tumours from patients that had metastases absent or present at diagnosis (Table 2). When the tumours from patients with metastases were split by tumour group (pT1/pT2 or pT3/pT4), no significant difference in COX2 expression was noted ($P=0.31$).

High (histoscore > 150) or low (histoscore < 150) COX2 expression was not related to the Gleason sum of the primary tumour ($P=0.54$), serum PSA level at diagnosis ($P=0.46$) or overall survival ($P=0.65$) in this patient cohort.

3.2. HER2 amplification and overexpression

No abnormalities of *HER2* gene copy number, chromosome 17 copy or *HER2* gene:chromosome ratio were observed in the BPH tissues. Only 8% (7/89) of cases of prostate cancer showed *HER2* gene amplification (ratio of *HER2*:chromosome 17 >2.0). All amplifications observed were low level (ratio L3), with the exception of one sample whose ratio was 10. In 39% of tumours, an increased copy number for *HER2* was observed, whilst in 47% of cases aneusomy of chromosome 17 was observed ($P<0.005$ and $P<0.005$ versus BPH, respectively) (Table 3). The frequency of altered *HER2* gene copy number, chromosome 17 copy number or *HER2* amplification was not significantly different between

Table 1
Patients' age and follow-up information

Pathology (number in group)	Median age in years (interquartile range)	Mean Gleason sum (SD)	Percentage death (Pca)	Mean follow-up (months) (SD)	Time to death (months) (SD)
BPH (28)	75 (71–75)	N/A	4 (1/28)	59 (10)	38 (7)
pT1 or pT2 (53)	64 (60–63)	6 (2)	30 (16/53)	66 (27)	44 (15)
ppT3 or pT4(36)	73 (63–76)	7 (2)	42 (15/36)	58 (24)	42 (26)
Non-metastatic (67)	64 (61–74)	6 (2)	28 (19/67)	68 (27)	44 (23)
Metastatic (22)	74 (68–80)	7 (1)	55 (12/22)	40 (33)	24 (6)

BPH, benign prostatic hyperplasia; N/A, not available. Percentage death = percentage of patients dying from prostate cancer during the follow-up period; Pca = number of prostate cancer-specific deaths. N.B. One BPH patient subsequently developed prostate cancer.

Table 2
COX2 Protein expression

	Median histoscore	Interquartile range	*P* value
BPH (28)	151	111–190	0.041
Tumour (86)	172	138–221	
pT1 or pT2 (49)	152	135–192	0.019
pT3 or pT4 (37)	207	155–242	
Non-metastatic (46)	170	135–226	0.62
Metastatic (22)	182	154–207	

P values represent differences between either BPH and all tumours, pT1/2 and pT3/4 cancers or metastatic versus non-metastatic cases at diagnosis by the Dunnett's *t*-test.

tumour groups (pT1/pT2 tumours or pT3/pT4 tumours) or between tumours from patients with and without metastases at diagnosis (Table 3). When tumours from patients with metastases were analysed by primary tumour stage (pT1/pT2 tumours or pT3/pT4 tumours), no significant difference was seen for the frequencies of abnormal *HER2* gene copy number, chromosome 17 copy number or *HER2* amplification ($P=0.2$, $P=0.68$ and $P=0.82$, respectively).

No significant differences were observed in the PSA levels at diagnosis, Gleason sum of the primary tumour or patient survival between tumours with or without aneusomy chromosome 17, abnormal *HER2* copy number or *HER2* amplification.

No BPH samples expressed HER2 protein and only 12% (11/89) of the tumour samples expressed the HER2 protein. All samples that expressed HER2 protein did so at very low levels (median histoscore 10, interquartile range 10–20). HER2 protein expression did not correlate with HER2 gene copy number or amplification ($P=0.63$ and $P=0.71$, respectively). Eleven samples had transitional cells, and 45 exhibited HER2 expression in the transitional cells, at levels higher than that seen in epithelial tumour cells (median histoscore of 80, interquartile range of 30–90).

Table 3
HER2 and chromosome 17 FISH results

	Chromosome 17	*HER2*	Amplified *HER2*
BPH (28)	0% (0)	0% (0)	0% (0)
Tumour (89)	47% (42)	39% (35)	8% (7)
P values	<0.005	<0.005	0.27
pT1 or pT2 (53)	51% (27)	32% (17)	8% (4)
ppT3 or pT4 (36)	42% (15)	50% (18)	8% (3)
P values	0.51	0.13	1.00
Non-metastatic (67)	43% (29)	42% (28)	6% (4)
Metastatic (22)	60% (13)	32% (7)	14% (3)
P values	0.29	0.56	1.00

FISH, fluorescent *in situ* hybridization.

Percentage of cases with polysomy 17 (chromosome 17), increased *HER2* copy number (*HER2*) or *HER2* gene amplification (amplified *HER2*), figures in brackets represent actual number of cases in each instance. *P* values represent differences between either BPH and all tumours, PpT1/2 and ppT3/4 cancers or metastatic versus non-metastatic cases at diagnosis by Fisher's exact test.

3.3. *HER2* and *COX2*

There was no correlation observed with either chromosome 17 copy number, *HER2* copy number or *HER2* amplification and COX2 expression ($P=0.4$, $P=0.36$ and $P=0.62$, respectively). However, tumours with an increased *HER2* copy number exhibited significantly higher COX2 expression than tumours with a normal HER2 copy number (Table 4). However, there was no significant difference in COX2 expression between tumours with and without *HER2* amplification or normal and abnormal chromosome 17 copy number (Table 4).

4. Discussion

Prostate cancer is the second most frequent cause of male cancer-related deaths in the United States of America (USA) and Western Europe [35]. Resistance to hormone therapy is related to a significant proportion of prostate cancer deaths with few therapeutic options available thereafter [36,37].

The prognostic significance of HER2 in breast cancer is now well established [18]. Controversy remains regarding the significance HER2 in prostate cancer. Our data showing *HER2* amplification in 8% of prostate cancers (using FISH and IHC methods used for HER2 diagnosis in breast cancer) suggests that HER2 is not involved in early prostate cancer and is in agreement with the data of Mark and colleagues who assessed *HER2* amplification using identical methods [17]. Ross

Table 4
COX2 expression compared with the FISH results

	Median	Interquartile range	*P* value
Chromosome 17 copy number			
Normal	178	135–221	0.601
Abnormal	170	142–219	
HER2 copy number			
Normal	155	135–195	0.034
Abnormal	195	146–230	
HER2 amplification			
Not amplified	170	137–220	0.757
Amplified	181	144–221	

Analysis of COX2 expression categorised by FISH results. No significant different in COX2 expression was observed between *HER2* amplified and non-amplified tumours, nor between chromosome 17 normosomic and aneusomic cases. However, COX2 expression was significantly higher in tumours with increased *HER2* copies (Dunnett's *t*-test).

and colleagues reported 41% of prostate tumours with an amplified *HER2* [20]. However, the FISH method employed only assessed the *HER2* copy number. We also found an elevated *HER2* copy number in 40% of the tumours, but when this was corrected for chromosome 17 aneusomy, the most of these cases were not amplified. Three other studies found no *HER2* amplification [30,31,38], although this may be due to sampling errors. Bubendorf and colleagues [31] disregarded amplification ratios of $>2<3.0$ (in contrast to current clinical diagnostic criteria), these criteria would have excluded 5/7 cases defined in this study. We conclude that *HER2* amplification is not a frequent event in primary prostate tumours. However, *HER2* amplification has been linked to the development of hormone-resistant prostate cancer [21].

HER2 protein expression was rarely seen in our cohort and was only ever present at very low levels. These results confirm data reported by Sanchez and colleagues and Jorda and colleagues, both of which used the DAKO HercepTest and reported a low level of HER2 expression in primary prostate tumours (2.6% and 15% weakly positive, respectively) [38,39]. We observed HER2 protein expression in 12% of tumours. However, this was never seen at a level above 1 plus staining ($>10\%$ of nuclei with weak positivity) if scored using the HercepTest criteria as used by Sanchez and colleagues [38]. They reported that when antigen retrieval was altered and the incubation time of the primary antibody was doubled, positive staining increased from 2.6% (1 specimen) to 40% (19 specimens) [38]. However, the clinical significance of this finding is unclear. This may explain why other studies using different antibodies and alternative antigen retrieval techniques have reported higher HER2 expression levels [26,27]. Extending epitope retrieval times sensitises the immunohistochemical method and allows the detection of lower levels of antigens. This would suggest that levels of HER2 expression in prostate cancer are indeed significantly lower than observed in breast and other tumour types. Therefore, unlike breast cancer, HER2 staining using the exact method outlined by the UK breast cancer HER2 diagnostic laboratories is usually of weak intensity, casting doubt on the usefulness of a HER2-targeted therapy for the treatment of primary prostate cancer. In addition, unlike breast cancer, *HER2* amplification or increased copy number does not appear to drive increased HER2 protein expression in this cohort. Even in the single case with a high level of *HER2* amplification, no significant HER2 expression was observed using the Herceptest. Recently, it has been suggested that HER2 is only commonly overexpressed in tumours following the development of resistance to hormonal therapy [28,32]. If this is proven to be the case, then HER2-targeted therapies may be of use in treating patients following the development of resistance. We are therefore currently investigating *HER2* expression in a cohort of 54 patients with tumour samples available both before and following the development of resistance.

Our study confirms that COX2 expression is elevated in prostate cancer [10,12,13] and that expression levels increase with tumour stage. Whilst Madann and colleagues reported COX2 expression is significantly higher in poorly differentiated tumours (Gleason scores 8–12) compared with well differentiated tumours (Gleason scores 1–3) [12], we observed no correlation to the Gleason sum in our study. No well differentiated tumours (Gleason score <3) were included and there was no significant difference in COX2 staining between moderately and poorly differentiated cancers in their study [12]. COX2 expression was normally distributed in the locally advanced tumour specimens (i.e. stage pT3 or pT4). pT1 or pT2 (organ-confined) tumours did not show an increased COX2 expression when compared with BPH. The increase in COX2 expression between tumour groups appears to be stage related and not related to grade or the presence of metastasis at diagnosis. The Gleason sum between the two tumour groups is was significantly different (Table 1) and COX2 expression was unaltered by the presence of metastasis (Table 2). Therefore, COX2 may be a potential therapeutic target for prostate cancer. COX2 inhibitors are currently in clinical trials for treatment of other solid tumours, e.g. colorectal cancer [9] and may have a role to play in the treatment of prostate cancer if given to the appropriate subset of patients with a high expression of this enzyme. It has been suggested that COX2 inhibitors could decrease angiogenesis, via decreased PGE2 and VEGF production [13]. We are currently investigating links between COX2 and angiogenesis in prostate cancer.

We found no correlation between COX2 expression and HER2 amplification or expression, suggesting that COX2 expression is not driven by HER2 in prostate cancer. COX2 expression is independent of HER2 expression in ovarian cancer [40] and recent evidence also suggests that the association in breast cancer is weaker that was previously thought to be the case [11,22]. It is possible that in prostate cancer another member of the HER family (possibly EGFR or EGFRvIII) is responsible for regulating COX2 expression. In summary, COX2-targeted therapies may have a role to play in the treatment of a subset of locally advanced prostate cancer patients. However, HER2 does not appear to regulate COX2 expression in this patient cohort and further investigations are necessary to establish what regulates COX2 expression in prostate cancer.

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References

1. Bakhle YS. COX2 and cancer: a new approach to an old problem. *Br J Pharmacol* 2001, **134**, 1137–1150.
2. Baek SJ, Wilson LC, Lee CH, Eling TE. Dual function of nonsteroidal anti-inflammatory drugs (NSAIDs): inhibition of cyclooxygenase and induction of NSAID-activated gene. *J Pharmacol Exp Ther* 2002, **301**, 1126–1131.
3. Kam PC, See AUL. Cyclooxygenase isoenzymes: physiological and pharmacological role. *Anaesthesia* 2000, **55**, 442–449.
4. Subbamaraiah K, Telang N, Ramonetti JT, *et al*. Transcription of cyclooxygenase-2 is enhanced in transformed mammary epithelial cells. *Cancer Res* 1996, **56**, 4424–4429.
5. Coffey RJ, Hawkey CJ, Damstrup L, *et al*. Epidermal growth factor receptor activation induces nuclear targeting of cyclooxygenase-2, basolateral release of prostaglandins and mitogenesis in polarising colon cancer cells. *Proc Nat Acad Sci USA* 1997, **94**, 657–662.
6. Vadlamudi R, Mandal M, Adam L, Steinbach G, Mendelsohn J, Kumar R. Regulation of cyclooxygenase-2 pathway by HER2 receptor. *Oncogene* 1999, **18**, 305–314.
7. Subbaramaiah K, Norton L, Gerald W, Dannenberg AJ. Cyclooxygenase-2 is overexpressed in HER-2/neu-positive breast cancer—evidence for involvement of AP-1 and PEA3. *J Biol Chem* 2002, **277**, 18649–18657.
8. Subbarayan V, Sabichi AL, Llansa N, Lippman SM, Menter DG. Differential expression of cyclooxygenase-2 and its regulation by tumor necrosis factor-alpha in normal and malignant prostate cells. *Cancer Res* 2001, **61**, 2720–2726.
9. Reddy BS, Hirose Y, Lubet R, *et al*. Chemoprevention of colon cancer by specific cyclooxygenase-2 inhibitor, celecoxib, administered during different stages of carcinogenesis. *Cancer Res* 2000, **60**, 293–297.
10. Madaan S, Abel PD, Chaudhary KS, Stamp GWH, Lalani EN. Over-expression of COX2 in human prostate cancer. *J Pathol* 2000, **190**, 44A.
11. Ristimaki A, Sivula A, Lundin J, *et al*. Prognostic significance of elevated cyclooxygenase-2 expression in breast cancer. *Cancer Res* 2002, **62**, 632–635.
12. Madaan S, Abel PD, Chaudhary KS, *et al*. Cytoplasmic induction and over-expression of cyclooxygenase-2 in human prostate cancer: implications for prevention and treatment. *Br J Urol Int* 2000, **86**, 736–741.
13. Kirschenbaum A, Liu XH, Yao S, Levine AC. The role of cyclooxygenase-2 in prostate cancer. *Urology* 2001, **58**, 127–131.
14. Liu XH, Kirschenbaum A, Yao S, Lee R, Holland JF, Levine AC. Inhibition of cyclooxygenase-2 suppresses angiogenesis and the growth of prostate cancer in vivo. *J Urol* 2000, **164**, 820–825.
15. Song XQ, Lin HP, Johnson AJ, Tseng PH, Yang YT, Kulp SK, Chen CS. Cyclooxygenase-2, player or spectator in cyclooxygenase-2 inhibitor-induced apoptosis in prostate cancer cells. *J Natl Cancer Inst* 2002, **94**, 585–591.
16. Johnson AJ, Song X, Hsu AL, Chen CS. Apoptosis signaling pathways mediated by cyclooxygenase-2 inhibitors in prostate cancer cells. *Adv Enzyme Regul* 2001, **41**, 221–235.
17. Mark HF, Feldman D, Das S, *et al*. Fluorescence in situ hybridization study of HER-2/neu oncogene amplification in prostate cancer. *Exp Mol Pathol* 1999, **66**, 170–178.
18. Bartlett JMS, Going JJ, Mallon EA, *et al*. Evaluating HER2 amplification and overexpression in breast cancer. *J Pathol* 2001, **195**, 422–428.
19. Masood S, Bui MM, Yung J-F, Mark HFL, Birkmeier JM, Hsu P. Reproducibility of LSI HEER-2/neu spectrum orange and CEP 17 spectrum green dual color deoxyribonucleic acid probe kit. *Ann Clin Lab Sci* 1998, **28**, 215–223.
20. Ross JS, Sheehan CE, Hayner-Buchan AM, *et al*. Prognostic significance of HER-2/neu gene amplification status by fluorescence in situ hybridization of prostate carcinoma. *Cancer* 1997, **79**, 2162–2170.
21. Craft N, Shostak Y, Carey M, Sawyers CL. A mechanism for hormone-independent prostate cancer through modulation of androgen receptor signaling by the HER-2/neu tyrosine kinase. *Nat Med* 1999, **5**, 280–285.
22. Witton CJ, Reeves JR, Going JJ, Cooke TG, Bartlett JM. Expression of the HER1-4 family of receptor tyrosine kinases in breast cancer. *J Pathol* 2003, **200**, 290–297.
23. Gu K, Mes-Masson AM, Gauthier J, Saad F. Overexpression of her-2/neu in human prostate cancer and benign hyperplasia. *Cancer Lett* 1996, **99**, 185–189.
24. Ware JL, Maygarden SJ, Koontz WWJ, Strom SC. Immunohistochemical detection of c-erbB-2 protein in human benign and neoplastic prostate. *Hum Pathol* 1991, **22**, 254–258.
25. Mellon K, Thompson S, Charlton RG, *et al*. p53, c-erbB-2 and the epidermal growth factor receptor in the benign and malignant prostate. *J Urol* 1992, **147**, 496–499.
26. Kuhn EJ, Kurnot RA, Sesterhenn IA, Chang EH, Moul JW. Expression of the c-erbB-2 (HER-2/neu) oncoprotein in human prostatic carcinoma. *J Urol* 1993, **150**, 1427–1433.
27. Sadasivan R, Morgan R, Jennings S, *et al*. Overexpression of Her-2/neu may be an indicator of poor prognosis in prostate cancer. *J Urol* 1993, **150**, 126–131.
28. Agus DB, Scher HI, Higgins B. Response of prostate cancer to anti-Her-2/neu antibody in androgen-dependent and-independent human xenograft models. *Cancer Res* 1999, **59**, 4761–4764.
29. Zhau HE, Wan DS, Zhou J, Miller GJ, von Eschenbach AC. Expression of c-erb B-2/neu proto-oncogene in human prostatic cancer tissues and cell lines. *Mol Carcinog* 1992, **5**, 320–327.
30. Fournier G, Latil A, Amet Y, *et al*. Gene amplifications in advanced-stage human prostate cancer. *Urol Res* 1995, **22**, 343–347.
31. Bubendorf L, Kononen J, Koivisto P, *et al*. Survey of gene amplifications during prostate cancer progression by high-throughout fluorescence in situ hybridization on tissue microarrays. *Cancer Res* 1999, **59**, 803–806.
32. Wen Y, Hu MC, Makino K, Spohn B, Bartholomeusz G, Yan DH, Hung MC. HER-2/neu promotes androgen-independent survival and growth of prostate cancer cells through the Akt pathway. *Cancer Res* 2000, **60**, 6841–6845.
33. Fraser J, Reeves JR, Stanton PD, *et al*. A Role for BRCA1 in sporadic breast cancer. *Br J Cancer* 2003, **88**, 1263–1270.
34. Bartlett JMS, Watters AD, Ballantyne SA, Going JJ, Grigor KM, Cooke TG. Is chromosome 9 loss a marker of disease recurrence in transitional cell carcinoma of the urinary bladder? *Br J Cancer* 1998, **77**, 2193–2198.
35. Agarwal R. Cell signaling and regulators of cell cycle as molecular targets for prostate cancer prevention by dietary agents. *Biochem Pharmacol* 2000, **60**, 1051–1059.
36. Dorkin TJ, Neal DE. Basic science aspects of prostate cancer. *Semin Cancer Biol* 1997, **8**, 21–27.
37. Oppenheimer JR. The pathologic examination of prostate tissue. Clinical review (internet): http://www.comed.com, 1999.
38. Sanchez KM, Sweeney CJ, Mass R, *et al*. Evaluation of HER2/neu Expression in prostatic adenocarcinomas. *Cancer* 2002, **85**, 1650–1655.
39. Jorda M, Morales A, Ghorab S, Fernandez G, Nadjo M, Block N. HER2 expression in prostatic cancer: a comparison with mammary carcinoma. *J Urol* 2002, **168**, 1412–1414.
40. Ferrandina G, Ranelletti FO, Lauriola L, *et al*. cyclooxygenase-2 (COX2), epidermal growth factor receptor (EGFR), and Her-2/neu expression in ovarian cancer. *Gynecol Oncol* 2002, **85**, 305–310.