SHORT REPORT

Adenoid cystic/basal cell carcinoma of the prostate strongly expresses HER-2/neu

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Adenoid cystic/basal cell carcinoma (ACBCC) is a rare neoplasm in the prostate. Definitive treatment is warranted, as among 19 patients previously reported by us, 5 had extraprostatic extension and 4 were metastatic. The *HER-2/neu* (*c-erbB-2*) gene has been reportedly overexpressed in adenoid cystic carcinomas in other organs, but its status in prostatic ACBCC was uncertain. Immunohistochemical staining and in situ hybridisation were carried out in 13 patients with ACBCC (11 from transurethral resection, 2 prostatectomy). One patient had metastasis to the lung. Citrate buffer and steam heat were used for antigen retrieval. Ten acinar adenocarcinomas of varying grades were also immunostained as controls. Protein and mRNA expression were 2+ to 3+ (of 3+) in all patients with ACBCC, compared to a breast cancer control with strong reactivity, whereas protein expression was noted in only one acinar carcinoma and mRNA expression was absent in all acinar carcinomas. Benign acini expressed *HER-2/neu* only in the basal layer. The finding of strong, consistent *HER-2/neu* expression in ACBCC suggests that treatment with Herceptin (trastuzumab) may be effective in patients with this rare tumour.

We previously described a rare neoplasm in the prostate, designated Adenoid cystic/basal cell carcinoma (ACBCC), combining predominant adenoid and basaloid histologies.¹ Although adenoid cystic carcinomas in some locations such as the breast have indolent

behaviour, this is not true in the prostate. Definitive treatment is warranted, as among 19 cases reported by us, 5 had extraprostatic extension and 4 were metastatic.¹ An additional four patients were recently reported, of whom three had no evidence of disease progression but one died of metastases to liver, lungs and bone 9 years after initial diagnosis.²

The *HER-2/neu* (*c-erbB-2*) gene is the human analogue of the rat *neu* gene in rat neuroblastomas. *HER-2/neu* is a membrane oncoprotein with tyrosine kinase activity, which functions as a growth factor receptor. It is amplified or overexpressed in 25–30% of invasive breast cancers in humans and is associated with an adverse clinical outcome. In acinar prostate carcinoma, *HER-2/neu* protein expression was infrequent³ or present in only some cases⁴ in several published studies. *HER-2/neu* expression has been described in salivary gland adenoid cystic carcinoma.⁵ The protein expression of *HER-2/neu* in the prostatic counterpart of this tumour was reported in one case in a recent report.⁶ Here, we studied the mRNA expression in this and other cases of ACBCC as well as the protein expression.

MATERIALS AND METHODS

Patients and specimens

Thirteen patients with ACBCC (age range 43–71 years) were studied. Eleven have been reported previously,¹ including the recently reported case 13.⁶ Eleven underwent transurethral resection and two prostatectomy. In addition to primary tumour, metastatic ACBCC from the lung was available for testing in one patient.

Figure 1 Case 4, with predominant basaloid pattern, shows 3+ membranous and cytoplasmic *HER-2/neu* reactivity, accentuated in all luminal cells of tumour nests; magnification 200×.

Figure 2 Case 1, with predominant adenoid pattern, has 2+, predominantly membranous *HER-2/neu* reactivity; magnification 200×.

Abbreviation: ACBCC, Adenoid cystic/basal cell carcinoma

Figure 3 Composite adenoid cystic/basal cell carcinoma (ACBCC) (bottom left) and acinar adenocarcinoma (top right) were present in the prostates of four patients. Case 12, magnification 200 $\times$.

Immunohistochemical staining

Immunostaining with monoclonal antibody to c-erbB-2 oncoprotein (HER-2/neu) (Zymed Laboratories, South San Francisco, California, USA) was carried out in 13 ACBCCs. Citrate buffer and steam heat were used for antigen retrieval as reported previously.⁶ Ten acinar adenocarcinomas of varying grades were also immunostained. HER-2 membranous staining was scored from 0 to 3+, in accordance with the HercepTest method. Patients with scores of 2+ to 3+ were considered to have HER-2 overexpression. HER-2 membrane immunoreactivity was observed in the basal cells of benign prostate acini, which served as internal controls to monitor the quality of immunohistochemical reaction in each patient. Another positive control was a patient with breast cancer who was HER-2/neu-positive. Negative controls comprised the application of non-immune whole rabbit serum to the tissue.

In situ hybridisation

The same cases used for immunostaining were also subjected to in situ hybridisation. An antisense DNA probe for HER-2/neu was designed (Invitrogen, Carlsbad, California, USA) as

follows: biotin-TGCCTGCTGCCCCGACCTGCT-biotin. A sense probe was designed in the reverse sequence. Sections from paraffin-wax-embedded tissue were floated in diethyl pyrocarbonate-treated water baths for slide preparation. In situ hybridisation was carried out as described previously.⁷ After deparaffinisation, tissue was digested with Proteinase K (Dako, Carpinteria, California, USA). The probe was diluted to a final concentration of 100 nM in CEP (consisting of sodium chloride, sodium citrate and dextran sulfate in formamide) hybridisation buffer (Vysis, Downers Grove, Illinois, USA) delivering 20 ng/slide, heated to 80°C to prevent secondary structure formation, cover slipped and hybridised at 37°C overnight. Two stringency washes were carried out using 2 $\times$ sodium chloride-trisodium citrate with 0.1% Nonidet P-40 at 73°C for 5 min. Slides were transferred to phosphate-buffered saline (pH 7.4) and the peroxide block was treated with 3% hydrogen peroxide in methanol for 10 minutes. Detection was accomplished using the tyramide signal amplification method of the GenPoint kit (Dako) with diaminobenzidine as chromogen.

RESULTS

Table 1 presents the clinical and pathological findings in the 13 ACBCCs. ACBCC showed patterns ranging from nests and trabeculae of basaloid cells (fig 1) to adenoid cystic-like patterns with focal cyst formation (fig 2). Most cysts had inspissated secretions. In all, 12 of 13 patients with ACBCC showed moderate (2+) to strong (3+) membranous HER-2/neu reactivity, in 50–100% of cells. Predominance of basaloid or adenoid pattern did not correlate with reactivity. Reactivity was usually stronger in the inner cells of tumour cell nests than in the peripheral cells (fig 1). Reactivity was noted in tumours with perineural and intraneural invasion. Moderate (2+) reactivity was seen in the pulmonary metastasis. Four of the 13 cases contained mixed ACBCC–acinar adenocarcinoma (fig 3). The acinar component was reactive only in case 12. However, the strong membranous and cytoplasmic reaction in ACBCC contrasted with weak reactivity in the acinar carcinoma component of this case (fig 4). In pure acinar adenocarcinomas, rare, weak reactivity was seen in only one of ten cases, and this was a high-grade tumour. Nine other cases were negative. Benign prostatic acini showed weak patchy reactivity of the basal layer, but secretory cells were negative. The breast cancer control had the usual membranous reactivity (fig 5).

Table 1 Clinical and pathologic findings of adenoid cystic–basal cell carcinoma in 13 patients

Patient no	Age (years)	Treatment	Staging	Concomitant acinar carcinoma	Tumour pattern (%)	Outcome	Her-2/neu protein strength (%reactive cells)
1	49	Exenteration	EPE* [†] ; lung, vertebral mets	No	70 A	DOD	2* (50)
2	81	TUR	bladder base* [‡] ; lung, corpus cavernosum mets	No	100 B	Unknown	2* (75) [§]
3	68	TUR	OC	No	80 A	AWD	2* (100) [§]
4	43	TUR, RP	EPE*	No	70 B	NED	3* (100) [§]
5	77	TUR	OC	No	100 B	NED	3* (100) [§]
6	87	TUR	OC	No	60 A	NED	2* (100)
7	74	TUR	OC	Yes*	95 B	Unknown	2*(100)
8	63	TUR, RP	OC	Yes [†]	90 B	NED	2* (70)
9	64	RP	OC	Yes [‡]	80 A	NED	Negative
10	81	TUR	OC	No	100 B	NED	2* (50) [§]
11	83	TUR	OC	No	100 B	NED	2* (50)
12	67	RP	OC	Yes*	100 B	NED	2* (100) [§]
13	71	TUR $\times$ 2	OC	No	100 B	D-other	3* (100) [§]

A, adenoid; AWD, alive with disease; B, basaloid; DOD, died of disease; D-other, died of other cause; EPE, extraprostatic extension; OC, organ-confined; NED, no evidence of tumour; RP, radical prostatectomy; TUR, transurethral resection.

*The two tumour types were diagnosed concomitantly.

[†]Acinar carcinoma was an incidental finding; no ACBCC on RP.

[‡]ACBCC was an incidental finding for RP carried out for acinar carcinoma.

[§]Inner cells of some nests had stronger reactivity compared with peripheral cells.

Figure 4 In case 12, weak reactivity in the acinar component (with perineural invasion, left) contrasted with the strong membranous and cytoplasmic reaction (right) in adenoid cystic/basal cell carcinoma (ACBCC); magnification 400 $\times$. Also, pure acinar carcinoma was non-reactive in 9/10 cases.

In situ hybridisation disclosed diffuse punctate cytoplasmic mRNA signal in all 13 patients with ACBCC (fig 6). In conventional acinar adenocarcinoma, punctate signal was absent or rare (fig 7). In benign acini, the basal cell distribution of mRNA signal paralleled that of the protein (fig 8). No relationship of protein or RNA expression of HER-2/neu to stage or metastatic status was evident, although the number of cases precluded statistical analysis.

DISCUSSION

ACBCC of the prostate, a tumour with the potential for extraprostatic extension, recurrence and metastasis,¹⁻⁸ consistently expressed HER-2/neu. The humanised monoclonal antibody Herceptin (trastuzumab) has been effective as a single agent and in combination chemotherapy in breast cancer. Its strong mRNA and protein expression in prostatic ACBCC suggests its use for this tumour, particularly in the setting of extraprostatic or metastatic tumours.

Others have studied HER-2/neu expression in prostatic acinar adenocarcinoma. Ross *et al* found that Her-2/neu

Figure 6 In situ hybridisation showing strong diffuse mRNA signal in adenoid cystic/basal cell carcinoma (ACBCC); magnification 200 $\times$.

amplification by the fluorescent in situ hybridisation technique correlated with disease recurrence, as did tumour grade and ploidy. However, by multivariate analysis, it was not important as an outcome predictor.⁹ In a subsequent study, using the standard HercepTest assay, almost no cases were positive. However, by modifying the technique to include an antigen retrieval citrate buffer with pH 9.0 and a lengthened, 1-h primary antibody incubation time, 50% of cases had some amount of reactivity.⁴ In that study, tumour grade correlated with degree of HER-2/neu expression, consistent with our finding that one high-grade acinar tumour was reactive.

The accentuated HER-2/neu reactivity in the inner cells of ACBCC tumour nests (fig 1) is of note. We established previously that the peripheral ACBCC cells predominantly reacted with cytokeratin 34 β E12, attesting to their basal cell phenotype, whereas luminal cells were reactive with cytokeratin 7.¹⁻⁶ This suggests that the cells with strongest HER-2/neu expression in ACBCC are those with some degree of differentiation towards the secretory cell phenotype compared with the peripheral, more myoepithelial-type cells that express S-100 protein and α -smooth muscle actin.¹⁻⁶

In other locations, HER-2/neu protein expression has been described in 58% of salivary gland adenoid cystic carcinomas²

Figure 5 Breast carcinoma control showing membranous reactivity for HER-2/neu; magnification 200 $\times$.

Figure 7 In situ hybridisation with rare HER-2/neu reactivity in Gleason 4+4=8 acinar adenocarcinoma; magnification 400 $\times$.

Figure 8 In situ hybridisation in benign epithelium; HER-2/neu mRNA is focal and confined to the basal layer; magnification 400 $\times$.

and in two of six dedifferentiated salivary gland and oral tumours. In the patients with salivary gland and oral tumours, its expression was restricted to the dedifferentiated component. Among tracheobronchial adenoid cystic carcinomas, tumours in only one of seven patients expressed HER-2/neu, but that patient developed distant metastases after surgery.¹⁰ In our study, HER-2/neu did not appear to correlate with histological pattern or outcome. HER-2/neu overexpression may be useful for treatment, however. In breast carcinoma, HER-2/neu gene amplification correlates best with response to Herceptin.¹¹ It would be of interest to carry out reverse transcriptase polymerase chain reaction to detect gene amplification in ACBCC, but the quantity of tissue in the available cases precluded this in our study. HER-2/neu overexpression does not always correlate with a response to treatment with Herceptin.¹² Nevertheless, the strong HER-2/neu overexpression uniquely prevalent in ACBCC suggests that treatment with Herceptin may be effective in patients with this rare tumour.

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