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Keratinocyte growth factor is a paracrine agent in human prostate cancer

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Introduction: Keratinocyte growth factor (KGF or fibroblast growth factor-7 [FGF-7]) is involved in development of the rat prostate, but its role in the human prostate is unclear. Its actions are mediated through interaction with its high-affinity receptor, namely the type IIIb isoform of the FGFR-1 (and not the IIIc isoform of FGFR-1).
Materials and methods: Using the reverse transcription-polymerase chain reaction (RT-PCR), expression of KGF and FGFR-1 (both the IIIb and IIIc isoforms) was examined in 17 snap-frozen prostate cancers (four grade 1, six grade 2 and seven grade 3) and six cases of BPH. The integrity of RNA was confirmed by RT-PCR of the house-keeping gene, glyceraldehyde 3-phosphate dehydrogenase. Using primary cultured prostate stroma and epithelium, the established prostate cancer cell line LNCaP, and SV_{40} transformed benign epithelial cells, the cell types responsible for KGF, FGFR-1IIIb and -IIIc expression were determined.
Results: Seven of 17 tumour samples expressed KGF, but no BPH samples expressed KGF. All 17 tumours expressed FGFR-1IIIb isoform mRNA, but none expressed the FGFR-1IIIc isoform. KGF was found only in stromal cells, but was not found in epithelium. FGFR-1IIIb isoform expression was identified in malignant and transformed epithelial cells.
Conclusions: We have shown the presence of KGF and its receptor in the human prostate and for the first time, have shown that it is found in stroma and that its receptor is found in epithelium, confirming its role as a potential paracrine agent in human prostate cancer.

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Over-expression of androgen-induced growth factor in human prostate cancer

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Introduction: Prostate cancer is androgen dependent and characterized by temporary regression after androgen ablation. Subsequent relapse may be related to abnormal expression of various peptide growth factors and their receptors. The androgen-induced growth factor (FGF-8) is part of the fibroblast growth factor family, but has not been studied in the human.
Materials and method: Using the non-radioactive digoxigenin system (Boehringer Mannheim, UK), *in situ* hybridization was used to detect FGF-8 transcripts and a sense-orientated riboprobe was included in each case in parallel experiments as an internal negative control. Formalin-fixed paraffin-embedded specimens from five cases of BPH and 31 newly diagnosed prostate cancers (16 grade 3, 10 grade 2 and five grade 1) were studied. Signals representing the presence of FGF-8 mRNA were assessed and graded into three groups (weak, moderate or strong).
Results: Twenty-two of the 31 prostate cancers (71%) expressed FGF-8 mRNA and its expression was uniform within individual tumours. High levels of FGF-8 expression were found more often in high-grade tumours (χ^2 test: $P < 0{\cdot}05$). None of the BPH samples expressed FGF-8. However, some areas of prostate epithelium adjacent to tumours expressed FGF-8 in basal cells at low to moderate intensity.
Conclusions: A significant up-regulation of FGF-8 expression was found in prostate cancer. Tissue from BPH was negative for FGF-8 expression, although epithelium adjacent to tumours was found to have localized low to moderate levels of FGF-8. High levels of FGF-8 may mediate androgen independence of prostate cancer.

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Heregulin as a potential autocrine growth factor and a poor prognostic indicator in prostate cancer

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Introduction: Heregulin (HRG), a new member of the epidermal growth factor family, binds to and activates the erbB-3 receptor (Science 256:1205, 1992; J. Biol. Chem. 269:14661, 1994). Their role in human prostate cancer is unknown. We report our original observations on the expression of HRG and erbB-3 proteins in human prostate cancer.
Materials and method: Using specific anti-peptide antibodies, 50 consecutive cases of prostate cancers (differentiation: well, moderate, poor; n = 8, 25 and 17, respectively) and four cases of BPH were immunostained for HRG and erbB-3 proteins. Representative areas from each case were used to assess HRG and erbB-3 expression.
Results: All four BPH cases did not express HRG or erbB-3. HRG overexpression was found in 36/50 cases of prostate cancer (72%). This overall expression rate did not differ between tumour grades. However, homogeneous cytoplasmic expression (> 90% of tumour cells positive) appeared to relate to high-grade tumours (well, moderate, poorly differentiated: 12·5%, 20%, 24%, respectively). Immunoreactivity for erbB-3 was found in 27 of 50 cases (54%). Strong homogeneous erbB-3 expression was observed in high-grade tumours (well, moderate, poorly differentiation: 0, 4, 18%, respectively). Thirteen cases had bony metastases; HRG and erbB-3 were absent in five of these cases, all of which responded to hormone manipulation and remained symptom-free at the time of review (follow-ups 2–4 years, mean 3). Despite hormone manipulation, the eight patients positive for HRG and/or erbB-3 were all dead at the time of review (follow-up 0·5 to 4 years, mean 2·5).
Conclusions: Heregulin may act as an autocrine growth factor in prostate cancer. Overexpression of HRG and/or erbB-3 may indicate a less favourable prognosis in advanced disease.

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Characterization of voltage sensitive Na+ channels in PC-3 cells by patch clamping is consistent with findings on immunohistochemistry, western blotting and FACS analysis

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Introduction: Functional voltage-sensitive Na^+ channels (VSSC) have been shown to be associated with invasion by Dunning MAT LyLu cells *in vitro* (*FEBS Letters* **369**: 290–294, 1995) and the PC-3 human prostate cancer cell line. The invasive phenotype of both PC-3 and MAT LyLu cells can be inhibited by blocking VSSC. In this study, we characterized further the VSSC by electrophysiology, immunohistochemistry, western blotting and fluorescent-activated cell sorting analysis (FACS).
Methods: (1) Electrophysiology: VSSC were analysed in PC-3 cells by whole-cell patch clamping to assess activation, inactivation and sensitivity to the highly specific VSSC antagonist, tetrodotoxin. (2) Immunohistochemistry: A three-step ABC method using a new polyclonal antibody to a highly conserved region in vertebrate VSSC was used to assess the number of positive cells and pattern of VSSC expression. (3) Western blotting: to assess size and potential glycosylation. (4) FACS: to determine whether channel expression was expressed on the surface and the correlation of positive cells with the electrophysiological findings.

Results: The tetrodotoxin dose–response curve was fitted by a sigmoidal logistic function, giving an IC_{50} of 4·8 (95% CI 0·18 to 5·87) nmol/L. Relative conductance of the VSSC fitted a Boltzmann function, giving half maximal conductance ($V_{1/2}$) at −15·2 (−20·4 to −9·9) mV and voltage sensitivity of 5·1 (3·3 to 7·0) mV per *e*-fold change in conductance (*k*). Immunohistochemistry and FACS analysis indicated that about 20% of cells expressed VSSC on the cell surface. The size of the VSSC was approximately 200kD on Western blotting.
Conclusions: The PC-3 VSSC is similar to VSSC found in excitable tissues such as neurones, although the relative molecular weight is slightly less, possibly because of reduced glycosylation. Slightly more cells were found on immunohistochemistry and FACS analysis to have VSSC, but overall they were broadly similar. This suggests that these techniques may be applied successfully in other cell lines to assess the presence of VSSC.

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Expression of membrane-type matrix metalloproteinase in malignant prostate compared with paired benign tissue and specimens of BPH

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Introduction: The metalloproteinases are a group of peptidases thought to be important in the process of cancer cell invasion and metastasis. A recently identified metalloproteinase, membrane-type matrix metalloproteinase (MT-MMP), has been shown to induce specific activation of pro-gelatinase A and enhance cellular invasion *in vitro*. Studies in lung tumour samples have shown increased expression compared with normal tissue and MT-MMP expression has been shown in colon, breast and head and neck carcinomas. We have assessed the relative expression of MT-MMP in paired benign and malignant prostate tissue and specimens of BPH.
Materials and methods: mRNA was extracted and cDNA synthesized from 23 specimens of histologically confirmed BPH, 40 specimens of prostate cancer and paired benign tissue from 19 of these. Cryodissection was used to ensure at least 70% tumour cell content in malignant samples, < 5% tumour cells in paired benign tissue and similar proportions of stroma and epithelium between samples. Expression was assessed using semi-quantitative RT-PCR with glyceraldehyde-3 phosphate dehydrogenase as an internal control.
Results: MT-MMP was expressed by all benign samples. The mean MT-MMP expression was significantly greater in malignant tissue compared with BPH ($P < 0·01$). Increased expression was associated with bone metastases in 14 of 17 cases. The mean expression was greater for benign tissue from prostates containing cancer than specimens of BPH but this did not reach statistical significance. In paired samples, 12 of 19 tumours showed increased expression compared with benign tissue and increased expression was demonstrated in seven of eight patients with metastatic disease. One case with a poorly differentiated metastatic tumour showed no MT-MMP expression. There was a weak association between increasing expression and increasing Gleason grade.
Conclusion: Prostate cancer is commonly associated with MT-MMP over-expression and there is a strong association between over-expression and metastatic disease.

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Expression of three candidate tumour suppressor genes on chromosome 5q, α-catenin, APC and MCC, in prostate cancer

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Introduction: Deletions of chromosome 5q have frequently been described in human prostate cancer and loss of heterozygosity at this site is associated with disease progression. Three candidate tumour suppressor genes lie in the region of common loss (5q21–22). These include, alpha catenin (α-cat), adenomatous polyposis coli (APC) and mutated in colorectal cancer (MCC). We have assessed the relative expression of each of these genes in paired benign and malignant prostatic tissue.
Materials and methods: Cryodissection was used to separate benign and malignant prostatic tissue from 19 patients. mRNA was extracted and cDNA synthesized using standard laboratory techniques. Gene expression was assessed using semi-quantitative RT-PCR with glyceraldehyde-3 phosphate dehydrogenase as an internal control. The number of PCR cycles was determined by plotting the reaction curve for each primer pair. Products were separated using gel electrophoresis, visualized with ethidium bromide and quanitified using computerized densitometry.
Results: α-cat expression was absent or reduced in malignant prostate compared with matched benign tissue in 10 of 19 cases. Aberrant expression was found in five of 11 non-metastatic cases and five of eight metastatic cases. MCC expression was reduced in only three of 19 patients all of which had locally advanced tumours and metastases on bone scan. APC expression was also reduced in three of 19 patients. Each of these cases had locally advanced disease and two had metastases.
Conclusion: Expression of the cell adhesion molecule α-cat was most frequently reduced (10 of 19 cases) supporting this gene as the candidate suppressor gene at this loci in prostate cancer. Reduced expression was associated with the presence of metastases. Reduction in expression of both APC and MCC was uncommon.