HUMAN ANDROGEN RECEPTOR ASSOCIATED HERIDITARY PROSTATE CANCER FACTOR AND ITS FUNCTION.

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INTRODUCTION. Prostate cancer has evolved as a major health problem in the male population of the Western world. It is the most commonly diagnosed malignancy and the second leading cause of cancer death. Most patients with early metastases are treated with androgen-deprivation therapy, and the consequence is a reduction of androgen-responsive cancer cells. Initially, about 70-80% of the patients respond to this therapy, but in the later stages, the tumor eventually becomes hormone-refractory and more aggressive, leading to a poor prognosis (1). Although the localized disease can be effectively treated with radical prostatectomy, no effective treatment is available for the hormone-refractory stage of the disease. Factors such as age, race, dietary, environmental factors, family history as well as hormonal factors have been involved in the etiology of prostate cancer. With increasing age, the production of steroid hormones changes, which may contribute to the favorable micro-environmental conditions for the development and progression of cancer (2).

METHOD. A human androgen receptor (AR) (amino acid 226-466) peptide was fused with pGEX-2TK vector and was expressed in Escherichia coli BL-21 (pLysS). The AR peptide was purified by immobilized GST-sepharose affinity chromatography and labeled with $\gamma^{32}\text{P}\text{[ATP]$ and protein kinase A. This$^{32}\text{P-GST-AR226-466}$ was used as bait in far-western blotting to screen an LNCaP human prostate cancer $\lambda\text{gt}11$ cDNA library. PolyA$^+$RNA was purified on oligo dT column and used to construct the cDNA expression library in $\lambda\text{gt}11$. Unamplified $\lambda\text{gt}11$ cDNA library was plated at a density of 20,000 pfu/100 mm agar plates (NZCYM Amp$^R$) with top agarose, incubated at 42°C for 4 h. The expression library was induced by isopropyl-$1\text{-thio-}\beta\text{-D-galactopyranoside (250 mM IPTG)$ by incubation for 3 h at 37°C. Only signals that were positive in the set of duplicates and not present in the GST control filters were considered for further analysis. $\lambda\text{gt}11$ phage DNA was purified from the positive clones for further characterization. From the initial 54 signals, 9 independent clones were isolated by two rounds of screening. The DNA sequence has been submitted to the GenBank.

RESULTS. We have isolated several human AR-associated factors by 2 hybrid screening, Sequences of the cDNAs were determined and deposited in Gene Bank. Subsequently other groups have identified the locus of this factor implicated in hereditary prostate cancer (HPCF). From human prostate cancer cells, several mutant HPCF were isolated and we now report the molecular characterization of this factor. Figure 1 shows the mRNA levels of HPCF in various tissues. We performed GST pull down assays using in vitro translated HPCF and GST hAR-AF-1 domains to show the specificity of interaction with AR. Affinity purified GST-HPCF was used to generate specific antibodies in rabbits and used to probe Western blots of cell extracts from LNCaP, PC-3, MCF-7 and HeLa cells. Direct immuno-fluoroescence studies showed that HPCF was exclusively nuclear in human prostate cancer cells and breast tumor cells. HPCF coprecipitated with AR in a ligand-dependent fashion when antibodies against either AR or HPCF
were used. Finally, RNASi-HPCF stable transfectants were used to infect human prostate cancer cells and colonies isolated and expanded. Cell death by apoptosis was confirmed in HPCF knock down cells by immunoprecipitation, annexin V and propidium bromide staining. There were 4 different translation products expressed in various cancer cells. The ratio of expression of these factors was also different. One of the mutants HPCF activated AR mediated transcription of a model gene in a constitutive manner thus implicating it as a ligand independent modulator of androgen receptor function in androgen independent prostate cancer growth.

DISCUSSION. Hereditary prostate cancer refers to a subtype of the disease in which Mendelian inheritance of a susceptibility gene is evident. This rare allele would account for 9% of all prostate cancer cases at ≤85 years of age, but could account for as much as 43% of prostate cancer cases diagnosed at ≤55 years of age. The notion that inherited susceptibilities play a role in the development of prostate cancer is supported by evidence for Mendelian segregation and by strong evidence for a familial effect on risk for the disease. HPCF represents a novel AR N-terminal-associated coactivator. HPCF enhances the transactivation function of AR, and this coactivity function is independent of androgen. In fact, HPCF has an LXXLL-like motif, which binds to the AR Ligand Binding Domain.

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REFERENCES.