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Review

Using Comparative Genomics to Leverage Animal Models in the Identification of Cancer Genes. Examples in Prostate Cancer

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Abstract. *The identification of cancer biomarkers that will predict susceptibility to disease and subsequent clinical outcome are key components of future genomics-based tailored medical care. Animal models of disease provide a rich resource for the identification of potential cancer biomarkers. Animal models of prostate cancer in particular offer the potential to identify cancer genes associated with dietary and environmental factors. The key issue is the timely and efficient identification of candidate genes that are likely to impact on human prostate cancer. Here, we demonstrate comparative genomics-based methods for the identification of candidate genes in animal models that are associated with human chromosomal regions implicated in prostate cancer. Using publicly available bioinformatics tools, comparisons can be made between cancer-specific datasets, genomic sequencing data and cross-species comparative maps to identify potential cancer biomarkers. This process is demonstrated by using rat models of prostate cancer to identify candidate human prostate cancer genes. Genes identified through these techniques can be screened as biomarkers for response to chemopreventive agents, as well as being used in transgenic or knockout mice to engineer better animal models of human prostate cancer. The bioinformatics techniques outlined here can be used to leverage genomic data from any animal cancer model for use in the study and treatment of human cancer.*

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The Use of Comparative Mapping in Gene Identification

The genomic sequencing of multiple species has led to extensive studies in comparative genomics (1-3). These studies have identified the relationship between genomes and defined syntenic regions for genomic similarity (4), thus laying the groundwork for their use in cancer research. Initial cancer studies have utilized comparative genomics to identify orthologs of cancer genes (5, 6), as exemplified for the Wnt signaling pathway (7, 8). Additional efforts have focused on the identification of syntenic regions for genetic mapping data in neuroblastoma and lung cancer animal models (9-11). Recently, experimental comparisons have been made across species at the level of whole transcriptomes using gene orthologs. Particular examples of this work include the analysis of mouse and human hepatocellular carcinoma (12, 13) and our own work in rat and human prostate cancer (14). Thus, the ability to use bioinformatics processes and tools has made it possible for researchers to examine and evaluate their cancer-specific genomic data from animal models for relevance in human cancer. Through simple publicly available bioinformatics tools for visualizing genomic information (genome browsers), an investigator can examine their cancer-specific genetic regions, expression results, and chromosomal region data against the backbone of the rat, mouse, or human genomes and, through cross-species comparative genomics, identify cancer genes based on multiple lines of scientific evidence. These local datasets, once studied and

published, can then be made publicly available, to be re-analyzed and expanded upon by subsequent scientists. Here, we outline a bioinformatics-based process in which datasets from animal models of prostate cancer can be examined and compared to human prostate cancer data using a Genome Browser. These datasets include rat prostate cancer microarray gene expression and genetic mapping studies, mouse transgenic and knockout data, rat mouse and human genomic sequence data, and comparative mapping data across the rat, mouse and human genomes. Through this process, comparative mapping can be used to identify syntenic regions of the rat genome that, in the human, are associated with prostate cancer risk. Within these regions, genes expressed in rat prostate tumors under specific environmental effects can be prioritized for validation studies.

Animal Models as a Source of Novel Cancer Genes

Animal models of cancer are important to the understanding of the pathophysiology of neoplasia and the development and testing of treatments. In particular, animal models are ideally suited for the examination of factors including diet, toxin exposures and drug responses, due to the ability to control the environmental exposures. This is important in the study of complex genetic traits such as the susceptibility to prostate cancer, with its associated environmental and genetic risks. Animal models of prostate cancer can be grouped into two general categories; i) genetically-engineered models, in which known cancer genes have been studied, and ii) spontaneous tumor models, where the genes involved in tumor risk are not known. Since our goal is the identification of novel cancer genes, we focused on the use of animal models in which the genes are not known. These types of studies can also be used in genetically-engineered animal models to identify additional genes that modify the tumor phenotype or outcome. For spontaneous models of prostate cancer, most work has been done on dog and rat models. These have been limited in their application to human prostatic disease because of their high cost, low tumor incidence and dissimilarities to prostate anatomy (15, 16). Yet, rat models have shown value in the study of hormonal-based carcinogenesis and because they mimic human responses to chemotherapeutics and dietary factors in prostatic neoplasia (17-19). In addition, the development of mouse prostate cancer models, using transgenic and knockout technologies, allows for the genetic manipulation and combination of prostate cancer genes to produce better models. This is exemplified by the development and interbreeding of genetically-engineered mouse prostate cancer models such as the NKX3.1, PTEN, p27 and Androgen receptor transgenic or knockout animals (20-25). Through the identification of additional genes in other animal models and their breeding into these mice, we can leverage the strength of the mouse model system to create better animal models for cancer research (26).

Genetic and Genomic Data in Animal Models of Cancer

Animal models offer the opportunity to collect large amounts of both genetic and genomic data associated with specific tumor characteristics. This has been demonstrated through the collection of gene expression microarray and genetic mapping data, both of which provide the opportunity to identify novel cancer genes. Genetic mapping in animal cancer models has been most extensively demonstrated using rat models of breast cancer (27-31). Prostate cancer genetic mapping studies are in their infancy, although preliminary mapping data has been generated for quantitative trait loci (QTLs) associated with prostate tumor formation and metastasis (Datta and Suckow, unpublished). Genomic studies have been more commonly performed and include studies of rat tumors under various conditions, for example aging and dietary changes (32-35). These datasets provide a rich source of candidate genes for subsequent validation.

Collecting Human Genomic and Genetic Cancer Data for Comparison

Animal data can be analyzed for relevance in human cancer by filtering it against human cancer genomic and genetic data. The sequencing of both the rat and mouse genomes allows for comparative mapping across species (2, 3). By linking the genomic cancer data across genomes, one can leverage syntenic mapping to prioritize genes found in animal studies for validation based on their location within chromosomal regions involved in human cancer. In order to accomplish this, one must have cancer-specific human genomic and genetic data. Specific sources include familial genetic mapping studies, cytogenetics and loss of heterozygosity studies, and comparative genomic hybridization and gene expression microarray studies. While some of these data types have been collected in central repositories such a CGAP (36), much still resides in individual publications and laboratory files. We have developed a data set of human chromosomal regions associated with prostate cancer, named ChromSorter PC (37) (Figure 1). This is a hand-annotated collection of data extracted from publications identified in automated searches of Medline using the key words "human", "prostate cancer" and "chromosome". The literature was examined, sorted and annotated for prostatic intraepithelial neoplasia, prostate cancer and prostatic metastasis. In each article, data was extracted regarding the experimental methods used (comparative genomic hybridization, loss of heterozygosity,

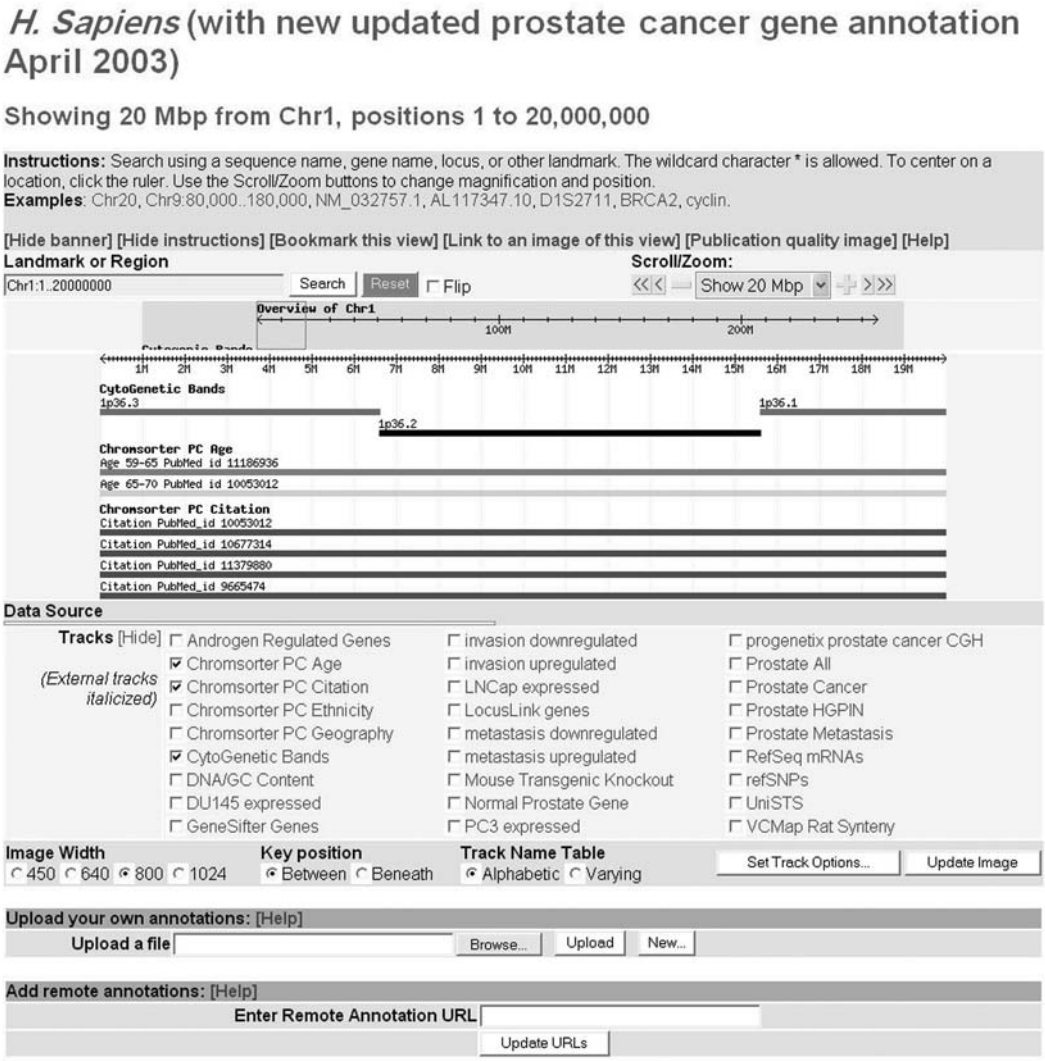

Figure 1. *ChromSorter PC data for human chromosome 1p36. Data is displayed on a generic genome browser (http://www.gmod.org). Each referenced chromosomal data element is displayed with respect to the human genome, and is linked to the original article in PubMed. The syntenic regions in the rat genome have been selected and are displayed at the bottom.*

karyotyping, chromosomal transfer), demographics (age groups, ethnicity) and chromosomal regions, including chromosomes, chromosomal arms and cytogenetic bands and, if available, genetic markers. These datasets, along with additional datasets for comparative genomic hybridization or prostate cancer gene expression studies, can be used as benchmarks for comparison with animal model data.

Integrating Local Data Using Large Scale Genomic Datasets

A crucial question is how specific cancer genes will be identified and sorted from the large quantities of genomic data. In this sorting process, bioinformatics-based comparative genomics will play a crucial role by associating genes with chromosomal regions that are linked to human cancer, thus making them strong candidates for further study. Bioinformatics and its associated computer-based tools allow for the integration and comparison of various types of genomic data including sequence, expression and functional information to cross entire genomes. The ability to rapidly organize this genomic data has been the hallmark of such bioinformatics and genomics pipelines as the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/), the University of California at Santa Cruz genome browser (http://genome.ucsc.edu/cgi-bin/hgGateway) and the Ensembl database (http://www.ensembl.org/). While

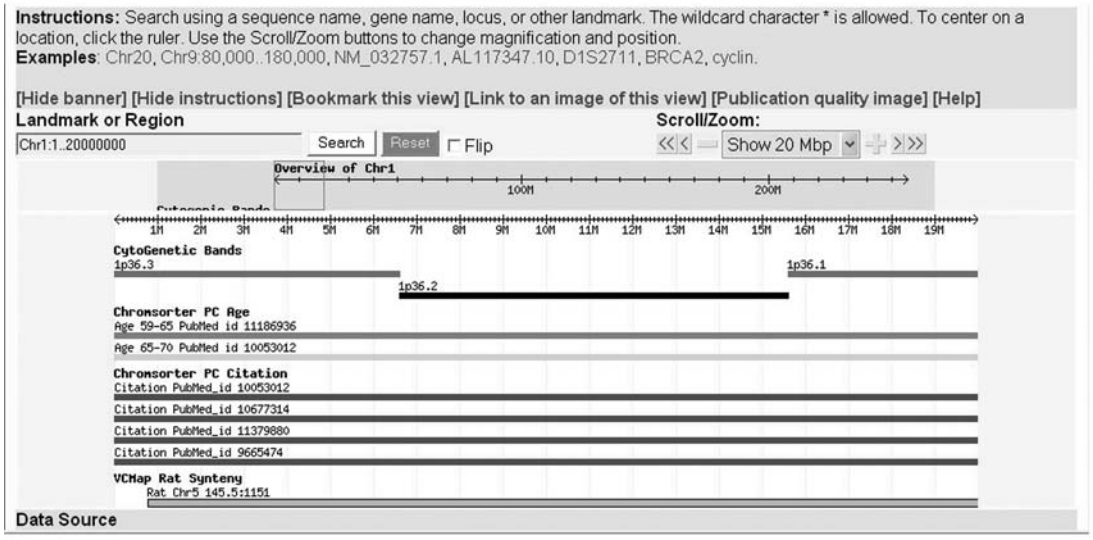

Figure 2. *Visualization of the human chromosome 1p36 region with synteny to rat chromosome 5.*

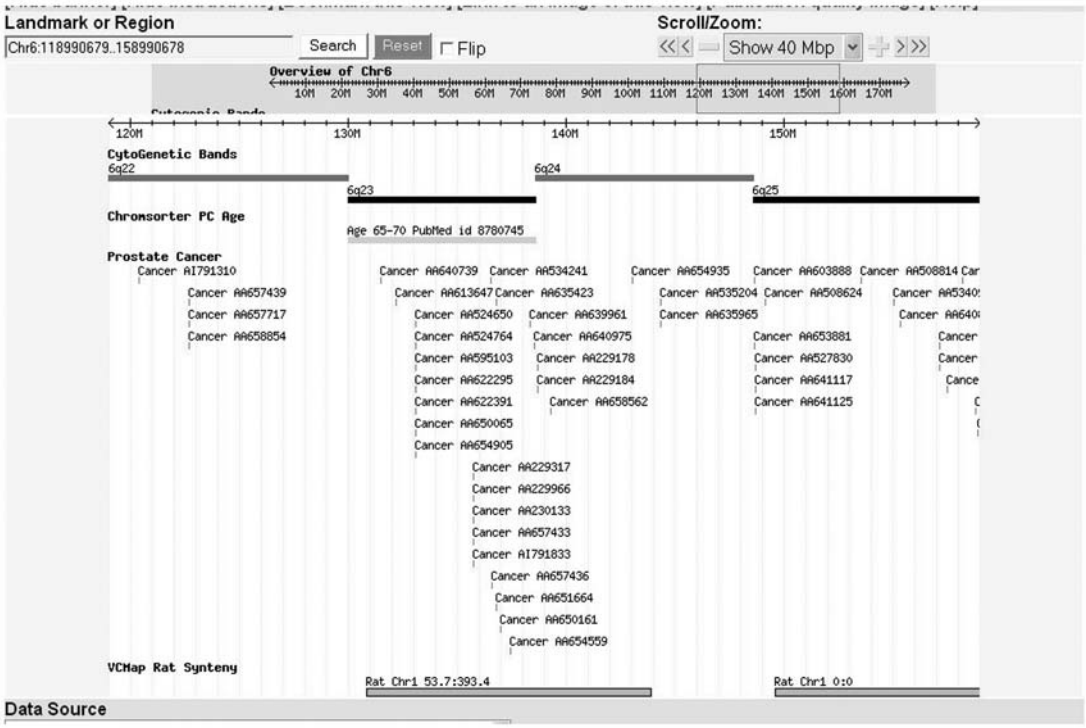

Figure 3. *Sorting of genes based on syntenic regions. Human chromosome 6q22-25 is shown. Human prostate cancer-expressed genes from CGAP are present as red bands. Genes that are located in the region defined by the light blue bands at the top (human prostate cancer reference) and bottom (genetic data in the rat model) are prioritized for study.*

extremely valuable in the analysis of genomic sequence data with respect to gene structure, function and even expression, the data presented is often very general and not focused on specific cancers. The true value of these Internet tools for cancer research is through the ability of users to upload their own locally generated cancer data for comparison with the stored genomic data. This provides a framework for comparing cancer data to known genes and

across genomes. The ability to upload and compare data is a feature that can be found on the UCSC genome browser. Some cancer-specific databases are now being developed that will allow for the analysis of cancer-specific data (CMAP, http://cmap.nci.nih.gov/), but currently do not have the ability to compare across species. For groups with biocomputing capabilities, the implementation and use of their own genome browsers allows for the collection of specific cancer-related data of relevance to the laboratory. This data can then be analyzed and reviewed without the need to reload datasets on a public genome browser. One particular tool we have used is the Generic Genome Browser developed by Lincoln Stein at Cold Spring Harbor Laboratories (http://stein.cshl.org/) and made publicly available through the Generic Model Organism Database Project (http://www.gmod.org/, Figures 1-3).

Using Cross-species Comparisons for Prostate Cancer Gene Identification

Animal data must be evaluated in the context of human cancer relevance. Thus, the identification and prioritization of genes identified in rat prostate cancer studies needs to be carried out in the context of human prostate cancer data. We have previously shown that gene expression profiles between rat and prostate cancer cells lines show a dramatic level of similarity (14). In addition, we have shown that, by concentrating on genes that demonstrate consistently similar changes to nutrients like Selenium across species, one can identify significant pathways involved in human prostate cancer (14). Here, we demonstrate how these studies can be extended through cross-species comparative mapping with genetic and genomic data. While demonstrated for prostate cancer, the techniques are applicable to any cancer system in which animal model data is available or can be generated.

Predicted Synteny Between Animal Model and Human Prostate Cancers

We have collected genetic data from a spontaneous rat model of prostate tumors. The Lobund-Wistar (L-W) rat is predisposed to spontaneously develop metastasizing tumors of the anterior prostate-seminal vesicle complex (38). While the anatomic similarity to human prostate cancer has been debated, the clinical similarities, including hormonal modulation, onset with age, similar chemosensitivity and dietary profiles, have been well established (38-41). As such, this model offers an opportunity to examine environmental effects in hormonal carcinogenesis. We have previously documented that the tumor susceptibility in the Lobund-Wistar rat can be transferred to a tumor-resistant Copenhagen rat strain through hybrid breeding, suggesting a genetic mechanism for tumor sensitivity (or resistance) (42). In a genetic characterization of the Lobund-Wistar colony using over 350 polymorphic genetic markers, it was noted that the animals in the Lobund-Wistar colony were 92% inbred over their genomes, with 8% of the genetic loci containing polymorphic (multiple) alleles. Using these regions with polymorphic markers, genotyping was performed on archival primary and metastatic tumors from over 50 Lobund-Wistar rats. In this genome sharing approach, we asked whether, in the polymorphic regions, one of the variant alleles preferentially segregated with tumor or metastasis formation. The results identified five chromosomal regions, two for tumor and three for metastasis. The regions identified include portions of rat chromosomes 1 and 18 for tumor formation, and areas on rat chromosomes 1, 8 and 10 for the metastatic spread of tumor. Comparative mapping between syntenic regions of the rat and human genomes reveal that these chromosomal regions in the Lobund-Wistar rat are syntenic to chromosomal regions involved in human prostate cancer. Using the human prostate cancer chromosomal data in the ChromSorter PC database (37), the chromosomal regions of the rat genome have predicted synteny to human chromosomes 1p36 (Figure 2) (43-45), 8q24 (46-50), 5q22-25 (51) and 6q22-27(Figure 3) (52-54), all areas that have been associated with prostate cancer by either familial genetic mapping, loss of heterozygosity studies and/or comparative genomic hybridization. These studies are currently being followed up by a full scale genetic backcross to better characterize the chromosomal regions.

Combining Synteny and Gene Expression Data to Leverage Animal Models for Prostate Cancer Gene Identification

In addition to these genetic studies, synteny data was used to overlay microarray gene expression data from rat prostate cancer cell lines treated with the chemopreventive agent Selenium on the human genome. In this way, the gene expression data from animal models can be sorted to prioritize genes of significance in human prostate cancer. The gene expression datasets were visualized across the rat and human genomes using a generic Genome Browser anchored to either human or rat genome sequence data. Visualization of the genomic data across species allows the syntenic alignment of data, thus leveraging the strengths of each species. Overlap was visually identified based on a concordance of gene expression microarray data and regions of synteny to human prostate cancer chromosomal regions. Examples of two genes are presented and include the endothelial-specific receptor tyrosine kinaseTie-2/Tek and the protein S100A4. Tie-2/Tek is present on human chromosome 9p21, a region involved in chromosomal aneuploidy in human prostate cancer (55, 56). This gene is

a receptor for angiopoietins and modulates the activity of vascular endothelial growth factor (VEGF) (57). It has been found to be elevated along with VEGF in the serum of prostate cancer patients (58, 59). An additional gene identified with respect to Selenium treatment is S100A4. This gene maps to Xq27-28, a region associated with human prostate cancer based on familial genetic mapping studies (60-63). S100A4 has recently been associated with prostate cancer (64, 65), along with multiple other members of the S100 protein family (66, 67). These genes, thus, become prioritized candidates for biomarker validation and use in the engineering of better animal models of prostate cancer.

Visualizing the Genomic Similarities of Animal Models to Human Disease, Now Possible with Available Bioinformatics Tools

Bioinformatics tools and processes demonstrate a new opportunity for scientists to perform cross-species syntenic analysis of their animal model genomic and genetic data to identify genes of significance in human cancer. Simple comparisons can be performed using Internet-enabled tools such as the UCSC Genome Browser, while local tools, such as the generic genome browser, are open source and can be modified and expanded by individual groups for more advanced comparative mapping studies. Through the publication and release of the associated data, further analysis can be achieved, thus advancing the field. These processes and the associated bioinformatics tools are freely available and can be applied to any disease process or animal model evaluation.

Using these tools, prostate cancer-specific genes from animal studies were selected as potential cancer biomarker candidates, based on their association with chromosomal region data in human prostate cancer. Thus, the process provides a novel method for identifying and prioritizing new cancer genes based on animal models. In this way, candidate gene identification can be accelerated. This data also demonstrates the applicability of prostate cancer animal models, in particular with dietary or chemoprevention studies, to identify genes related to nutrients and prostate cancer. This current method also has the capability of analyzing other animal models of human disease at the genomic level, identifying both common and differing features that indicate the strengths and weaknesses of the model. Subsequent selection of specific genes or regions for breeding and genetic manipulation will allow for the development of better animal models of cancer.

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References

1 Pennacchio LA: Insights from human/mouse genome comparisons. Mamm Genome *14(7)*: 429-436, 2003.
2 Gibbs RA *et al*: Genome sequence of the Brown Norway rat yields insights into mammalian evolution. Nature *428(6982)*: 493-521, 2004.
3 Waterston RH *et al*: Initial sequencing and comparative analysis of the mouse genome. Nature *420(6915)*: 520-562, 2002.
4 Rubin GM *et al*: Comparative genomics of the eukaryotes. Science *287(5461)*: 2204-2215, 2000.
5 Pickeral OK *et al*: Classical oncogenes and tumor suppressor genes: a comparative genomics perspective. Neoplasia *2(3)*: 280-286, 2000.
6 Makalowski W, Zhang J and Boguski MS: Comparative analysis of 1196 orthologous mouse and human full-length mRNA and protein sequences. Genome Res *6(9)*: 846-857, 1996.
7 Katoh M: Comparative genomics on *Wnt7a* orthologs. Oncol Rep *13(4)*: 777-780, 2005.
8 Katoh M: Comparative genomics on *Wnt8a* and *Wnt8b* genes. Int J Oncol *26(4)*: 1129-1133, 2005.
9 Yanagitani N *et al*: Localization of a human lung adenocarcinoma susceptibility locus, possibly syntenic to the mouse Pas1 locus, in the vicinity of the D12S1034 locus on chromosome 12p11.2-p12.1. Carcinogenesis *23(7)*: 1177-1183, 2002.
10 Lastowska M *et al*: Regions syntenic to human 17q are gained in mouse and rat neuroblastoma. Genes Chromosomes Cancer *40(2)*: 158-163, 2004.
11 Corso C and Parry JM: Comparative genomic hybridization analysis of N-methyl-N'-nitrosoguanidine-induced rat gastrointestinal tumors discloses a cytogenetic fingerprint. Environ Mol Mutagen *43(1)*: 20-27, 2004.
12 Lee JS, Grisham JW and Thorgeirsson SS: Comparative functional genomics for identifying models of human cancer. Carcinogenesis, 2005.
13 Lee JS *et al*: Application of comparative functional genomics to identify best-fit mouse models to study human cancer. Nat Genet *36(12)*: 1306-1311, 2004.
14 Schlicht M *et al*: Cross-species global and subset gene expression profiling identifies genes involved in prostate cancer response to selenium. BMC Genomics *5(1)*: 58, 2004
15 Lucia MS *et al*: Workgroup I: rodent models of prostate cancer. Prostate *36(1)*: 49-55, 1998.
16 Waters DJ and Bostwick DG: The canine prostate is a spontaneous model of intraepithelial neoplasia and prostate cancer progression. Anticancer Res *17(3A)*: 1467-1470, 1997.
17 Pollard M and Wolter W: Prevention of spontaneous prostate-related cancer in Lobund-Wistar rats by a soy protein isolate/isoflavone diet. Prostate *45(2)*: 101-105, 2000.
18 Pollard M, Wolter W and Sun L: Diet and the duration of testosterone-dependent prostate cancer in Lobund-Wistar rats. Cancer Lett *173(2)*: 127-131, 2001.
19 Weber KS *et al*: Dietary soy-phytoestrogens decrease testosterone levels and prostate weight without altering LH, prostate 5alpha-reductase or testicular steroidogenic acute regulatory peptide levels in adult male Sprague- Dawley rats. J Endocrinol *170(3)*: 591-599, 2001.
20 Di Cristofano A *et al*: Pten and p27KIP1 cooperate in prostate cancer tumor suppression in the mouse. Nat Genet *27(2)*: 222-224, 2001.

21 Gao H *et al*: A critical role for *p27kip1* gene dosage in a mouse model of prostate carcinogenesis. Proc Natl Acad Sci USA *101(49)*: 17204-17209, 2004.

22 Kim MJ *et al*: Cooperativity of Nkx3.1 and Pten loss of function in a mouse model of prostate carcinogenesis. Proc Natl Acad Sci USA *99(5)*: 2884-2889, 2002.

23 Kwabi-Addo B *et al*: Haploinsufficiency of the *Pten* tumor suppressor gene promotes prostate cancer progression. Proc Natl Acad Sci USA *98(20)*: 11563-11568, 2001.

24 Podsypanina K *et al*: Mutation of Pten/Mmac1 in mice causes neoplasia in multiple organ systems. Proc Natl Acad Sci USA *96(4)*: 1563-1568, 1999.

25 Tanaka M *et al*: Nkx3.1, a murine homolog of *Drosophila bagpipe,* regulates epithelial ductal branching and proliferation of the prostate and palatine glands. Dev Dyn *219(2)*: 248-260, 2000.

26 Powell WC *et al*: Mouse strains for prostate tumorigenesis based on genes altered in human prostate cancer. Curr Drug Targets *4(3)*: 263-279, 2003.

27 Harvell DM *et al*: Diet-gene interactions in estrogen-induced mammary carcinogenesis in the ACI rat. J Nutr *131(11 Suppl)*: 3087S-3091S, 2001.

28 Shull JD *et al*: Susceptibility to estrogen-induced mammary cancer segregates as an incompletely dominant phenotype in reciprocal crosses between the ACI and Copenhagen rat strains. Endocrinology *142(12)*: 5124-5130, 2001.

29 Shepel LA and Gould MN: The genetic components of susceptibility to breast cancer in the rat. Prog Exp Tumor Res *35*: 158-169, 1999.

30 Lan H *et al*: Genetic loci controlling breast cancer susceptibility in the Wistar-Kyoto rat. Genetics *157(1)*: 331-339, 2001.

31 Gould KA *et al*: Genetic determination of susceptibility to estrogen-induced mammary cancer in the ACI rat: mapping of Emca1 and Emca2 to chromosomes 5 and 18. Genetics *168(4)*: 2113-2125, 2004.

32 Jiang F and Wang Z: Identification of androgen-responsive genes in the rat ventral prostate by complementary deoxyribonucleic acid subtraction and microarray. Endocrinology *144(4)*: 1257-1265.

33 Reyes N *et al*: Microarray analysis of diet-induced alterations in gene expression in the ACI rat prostate. Eur J Cancer Prev *11(Suppl 2)*: S37-42, 2002.

34 Reyes I *et al*: Aging-associated changes in gene expression in the ACI rat prostate: implications for carcinogenesis. Prostate *63(2)*: 169-186, 2005.

35 Desai KV *et al*: Gene expression profiling identifies a unique androgen-mediated inflammatory/immune signature and a PTEN (phosphatase and tensin homolog deleted on chromosome 10)-mediated apoptotic response specific to the rat ventral prostate. Mol Endocrinol *18(12)*: 2895-2907, 2004.

36 Strausberg RL: The Cancer Genome Anatomy Project: new resources for reading the molecular signatures of cancer. J Pathol *195(1)*: 31-40, 2001.

37 Etim A *et al*: ChromSorter PC: a database of chromosomal regions associated with human prostate cancer. BMC Genomics *5(1)*: 27, 2004.

38 Pollard M: Lobund-Wistar rat model of prostate cancer in man. Prostate *37(1)*: 1-4, 1998.

39 Pollard M, Wolter W and Sun L: Prevention of induced prostate-related cancer by soy protein isolate/isoflavone-supplemented diet in Lobund-Wistar rats. In Vivo *14(3)*: 389-392, 2000.

40 Pollard M, Wolter W and Sun L: Diet and the duration of testosterone-dependent prostate cancer in Lobund-Wistar rats. Cancer Lett *173(2)*: 127-131, 2001.

41 Pollard M and Luckert PH: Influence of isoflavones in soy protein isolates on development of induced prostate-related cancers in L-W rats. Nutr Cancer *28(1)*: 41-45, 1997.

42 Suckow MA, Wolter W and Pollard M: Susceptibility of Lobund-Wistar x Copenhagen hybrid rats to autochthonous prostate carcinogenesis. Prostate, 2005.

43 Gibbs M *et al*: Evidence for a rare prostate cancer-susceptibility locus at chromosome 1p36. Am J Hum Genet *64(3)*: 776-787, 1999.

44 Berry R *et al*: Linkage analyses at the chromosome 1 loci 1q24-25 (HPC1), 1q42.2-43 (PCAP), and 1p36 (CAPB) in families with hereditary prostate cancer. Am J Hum Genet *66(2)*: 539-546, 2000.

45 Badzioch M *et al*: Suggestive evidence for a site specific prostate cancer gene on chromosome 1p36. The CRC/BPG UK Familial Prostate Cancer Study Coordinators and Collaborators. The EU Biomed Collaborators. J Med Genet *37(12)*: 947-949, 2000.

46 Porkka KP *et al*: RAD21 and KIAA0196 at 8q24 are amplified and overexpressed in prostate cancer. Genes Chromosomes Cancer *39(1)*: 1-10, 2004.

47 Tsuchiya N *et al*: Mapping and gene expression profile of the minimally overrepresented 8q24 region in prostate cancer. Am J Pathol *160(5)*: 1799-1806, 2002.

48 Tsuchiya N *et al*: Clinical significance of alterations of chromosome 8 detected by fluorescence *in situ* hybridization analysis in pathologic organ-confined prostate cancer. Genes Chromosomes Cancer *34(4)*: 363-371, 2002.

49 van Dekken H *et al*: Genetic evaluation of localized prostate cancer in a cohort of forty patients: gain of distal 8q discriminates between progressors and nonprogressors. Lab Invest *83(6)*: 789-796, 2003.

50 Van Den Berg C *et al*: DNA sequence amplification in human prostate cancer identified by chromosome microdissection: potential prognostic implications. Clin Cancer Res *1(1)*: 11-18, 1995.

51 Balint I *et al*: Cloning and characterisation of the RBCC728/TRIM36 zinc-binding protein from the tumor suppressor gene region at chromosome 5q22.3. Gene *332*: 45-50, 2004.

52 Chu LW *et al*: Genetic markers useful for distinguishing between organ-confined and locally advanced prostate cancer. Genes Chromosomes Cancer *36(3)*: 303-312, 2003.

53 Hyytinen ER *et al*: Defining the region(s) of deletion at 6q16-q22 in human prostate cancer. Genes Chromosomes Cancer *34(3)*: 306-312, 2002.

54 Wang Z, Lai FM and Zhang J: Analysis of loss of heterozygosity on chromosome 6 in human prostate carcinoma and prostatic intraepithelial neoplasia. Zhonghua Bing Li Xue Za Zhi *30(6)*: 414-417, 2001.

55 Dong JT: Chromosomal deletions and tumor suppressor genes in prostate cancer. Cancer Metastasis Rev *20(3-4)*: 173-193, 2001.

56 Heidenreich B *et al*: Aneuploidy of chromosome 9 and the tumor suppressor genes p16(INK4) and p15(INK4B) detected by *in situ* hybridization in locally advanced prostate cancer. Eur Urol *38(4)*: 475-482, 2000.

57 Merenmies J, Parada LF and Henkemeyer M: Receptor tyrosine kinase signaling in vascular development. Cell Growth Differ *8(1)*: 3-10, 1997.

58 Caine GJ *et al*: Plasma angiopoietin-1, angiopoietin-2 and Tie-2 in breast and prostate cancer: a comparison with VEGF and Flt-1. Eur J Clin Invest *33(10)*: 883-890, 2003.
59 Wurmbach JH *et al*: The expression of angiopoietins and their receptor Tie-2 in human prostate carcinoma. Anticancer Res *20(6D)*: 5217-5220, 2000.
60 Xu J *et al*: Evidence for a prostate cancer susceptibility locus on the X chromosome. Nat Genet *20(2)*: 175-179, 1998.
61 Peters MA *et al*: Genetic linkage analysis of prostate cancer families to Xq27-28. Hum Hered *51(1-2)*: 107-113, 2001.
62 Kibel AS *et al*: Xq27-28 deletions in prostate carcinoma. Genes Chromosomes Cancer *37(4)*: 381-388, 2003.
63 Farnham JM *et al*: Confirmation of the HPCX prostate cancer predisposition locus in large Utah prostate cancer pedigrees. Hum Genet *116(3)*: 179-185, 2005.
64 Gupta S *et al*: Differential expression of S100A2 and S100A4 during progression of human prostate adenocarcinoma. J Clin Oncol *21(1)*: 106-112, 2003.
65 Saleem M *et al*: Prognostic significance of metastasis-associated protein S100A4 (Mts1) in prostate cancer progression and chemoprevention regimens in an autochthonous mouse model. Clin Cancer Res *11(1)*: 147-153, 2005.
66 Rehman I *et al*: S100A6 (Calcyclin) is a prostate basal cell marker absent in prostate cancer and its precursors. Br J Cancer *91(4)*: 739-744, 2004.
67 Rehman I *et al*: Dysregulated expression of S100A11 (calgizzarin) in prostate cancer and precursor lesions. Hum Pathol *35(11)*: 1385-1391, 2004.

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