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Molecular evolution of the prostate cancer susceptibility locus *RNASEL*: Evidence for positive selection

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Abstract

Recent research implicates viral infection as a factor that may contribute to the risk of prostate cancer. Allelic variation at the *RNASEL* locus is associated with the risk of infection by a newly discovered retrovirus called XMRV, and with hereditary risk of prostate cancer. This evidence suggests that the *RNASEL* locus has undergone antagonistic coevolution with the retrovirus over evolutionary time. If this is the case, then both the *RNASEL* locus and the retrovirus should show evidence of positive selection. Here we use molecular-evolutionary methods to investigate the prediction that the *RNASEL* locus will exhibit evidence of positive selection. We find evidence that positive selection has acted on this locus over evolutionary time. We further find, using a Bayesian estimation procedure, that Asp541Glu, which was found to be associated with prostate cancer risk in Caucasians in a recent meta-analysis, shows an elevated probability of positive selection. Previous studies provide evidence for rapid evolution of the infection-mediating *gag* gene in the XMRV retrovirus. Taken together, these results suggest that antagonistic coevolution may have occurred between a specific host locus involved in immune defense (*RNASEL*) and a viral pathogen. In turn, genetic variation associated with this apparent coevolution may influence susceptibility to prostate cancer.

Keywords: XMRV; Prostate cancer; Virus; *RNASEL*; Coevolution; Positive selection

1. Introduction

A substantial body of evidence links specific parasitic infections to cancer risk and progression in a variety of tissues (e.g. breast cancer: Pogo and Holland, 1997; colon cancer: Laghi et al., 1999). The mechanisms that cause associations between infection and cancer are still being elucidated. In some cases, the infection and the associated interactions between parasites and the immune system can cause chronic tissue damage and repeated tissue repair that increase the risk of cancer. In other cases, viruses may activate mechanisms that prevent apoptosis, or increase cell replication rates, to enhance their own replication (Barber, 2001). These adaptations on the part of viruses can have the secondary effect of promoting the development and survival of cancer cell lineages, again increasing the risk of cancer (Persing and Prendergast, 1999; Barber, 2001; Ewald, 2000).

Recently, evidence has been found that implicates viral infection in the risk of prostate cancer (reviewed by Wagenlehner et al., 2007) mediated by *RNASEL*, the long sought-after Hereditary Prostate Cancer 1 gene (Carpten et al., 2002; reviewed by Silverman, 2007). Research involving the hybridization of RNA from prostate tumors of individuals bearing different genotypes at amino acid position 462 of the *RNASEL* locus to a microarray representing conserved sequences from a wide range of viruses identified a novel gammaretrovirus (XMRV) related to xenotropic murine leukemia viruses (Urisman et al., 2006). There were two alleles involved, arginine (R) and glutamine (Q), with the former being common relative to the latter. A high proportion (40%) of the tumors from individuals homozygous for the susceptible version of the 462 allele (QQ) harbored the genome of XMRV, whereas few of the tumors (2%) from heterozygous or homozygous wildtype (RR) genotypes showed any evidence for the presence of the retrovirus. These findings indicate that the XMRV virus interacts with prostate cells and may increase the risk of cancer in individuals genetically susceptible due to their genotype at the *RNASEL* locus.

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Urisman et al. (2006) sequenced multiple copies of several different genes in the XMRV viral genome (*gag*, *gag-pro-pol*, and *env*) and compared them, using phylogenetic analysis, to other MuLVs. The XMRV sequences formed a strongly supported clade, which was the sister clade to an MuLV clade-containing viral strains NFS-Th-1 and NZB-9-1. Analysis of the *gag* leader sequence of the *gag* gene of XMRV showed high levels of sequence variation consistent with the possibility of positive selection (Urisman et al., 2006), and studies of this region in closely related MuLVs implicate it as an important mediator of infectivity and virulence (Corbin et al., 1994; Fujisawa et al., 1998).

The evidence linking genotypic variation at the *RNASEL* locus with susceptibility to viral infection suggests that this gene may be subject to antagonistic coevolution with XMRV, such that different genetic strains of the parasite are evolving over time to exploit the host, and host loci are evolving in response. This type of antagonistic coevolution should leave a molecular evolutionary signature in both the host and parasite genomes across evolutionary time, as for diverse other genes involved in host-parasite evolutionary dynamics (Yang and Bielawski, 2000). Specifically, this type of coevolution is expected to leave a signal of positive (diversifying) selection, where new protein variants are selected at such a high rate that the rate of non-synonymous substitution exceeds the rate of synonymous substitution, controlled for opportunity (Hughes, 1999; Yang and Bielawski, 2000).

In this paper, we use methods from molecular evolutionary genetics designed to test for the signal of diversifying selection across species in a phylogenetic context. We use these methods to search for the signal of diversifying selection in the *RNASEL* gene, to test the prediction that this gene has been involved in antagonistic coevolution with pathogens.

RNASEL codes for the RNase L protein, a constitutively expressed latent endoribonclease that mediates the IFN-inducible 2–5A system (Li and Tai, 2006). It functions (in part) as a tumor suppressor, acting to induce apoptosis of cancer cells (Malathi et al., 2004), and is also associated the activity of interferons (Zhou et al., 1997). RNase L appears to implement antiviral effects through several different mechanisms, including cleavage of viral RNA, prevention of viral protein synthesis through destruction of rRNA, induction of apoptosis and induction of other antiviral genes (Silverman, 2007).

An association of *RNASEL* with hereditary prostate cancer has been found in a variety of studies (e.g. Carpten et al., 2002; Rennert et al., 2002), although other studies have found no association (e.g. Downing et al., 2003). A recent meta-analysis supported a role for polymorphism in the *RNASEL* gene in the development of prostate cancer (particularly the Asp541Glu polymorphism), but there was substantially heterogeneity among studies (Li and Tai, 2006). In this paper, we analyze sequence variation in the DNA sequences of this gene across multiple species, to determine if *RNASEL* shows signs of positive selection over evolutionary time. This analysis provides a preliminary test of the hypothesis that the molecular evolution of the *RNASEL* gene has been driven in part by host-viral antagonistic coevolution.

2. Methods

The program CODEML in the PAML computer program package (Yang, 1997) implements maximum-likelihood-based methods that test for a statistical excess of non-synonymous nucleotide changes (leading to amino acid substitutions), over synonymous changes, in sequence data of protein coding genes.

DNA sequences for the *RNASEL* genes from different mammals were obtained from GenBank. First, the mouse or human sequence was obtained by searching the peptide database. This protein was then used for a translated BLAST search (tblastn). Genes with the same name with an e score less than $1 \times e^{-20}$ were assumed to be orthologous. We identified eight sequences appropriate for analysis: mouse (NM011882), rat (NM182673), dog (XM547430), cow (XR027398), macaque (NM001042433), human (NM021133), orangutan (CR858878) and chimpanzee (XM524990). DNA sequences from GenBank were saved in Fasta format and analyzed with the programs Clustal X (Thompson et al., 1997) and RevTrans (Wernersson and Pedersen, 2003). Sequences were imported into RevTrans in Fasta format and translated. The amino acid sequences were aligned in Clustal X using the multiple alignment parameters 'Gap opening penalty = 15.00, Gap extension penalty = 35.00'. The Gonnet matrix was used to characterize amino acid similarities. Several different gap opening and extension penalties were tried to optimize the alignment. The alignment was also inspected by eye and further adjusted. The final peptide alignment was then used in RevTrans as a ''guide alignment''. The DNA sequences were translated, aligned to match the guide alignment, and reverse translated to give the final DNA sequence alignment.

The aligned sequences were imported into the phylogenetic analysis program PAUP 4.0b10 (Swofford, 2003). An unweighted heuristic search with TBR and 10 random addition-order replicates was used to identify the most parsimonious tree, which was used as a base topology to calculate the following parameters via maximum likelihood: base frequencies, transition/transversion ratio, gamma parameter (alpha), and proportion of invariant sites. These estimates were then used to parameterize a maximum likelihood analysis of the phylogenetic relationships of the sequences involved. A heuristic search was used, with TBR and 10 random addition-order replicates. The tree obtained agreed with previous estimates of the relationships among the taxa involved based on multiple datasets (Springer et al., 2004). The tree from PAUP was imported into TreeView (Page, 2000) and edited to insure Q1 that it was an unrooted tree appropriate for analysis with PAML (Yang, 1997).

Analyses of d*N*/d*S* ratios (ω) were carried out with the CODEML program in PAML 3.15 (Yang, 1997), using the sequence alignments from Clustal X and RevTrans, and the tree from PAUP. We used the N-sites model, which uses codons as sites and allows ω to vary among sites (Nielsen and Yang, 1998). We also used a lineage-specific model to investigate selection on the human lineage specifically (Yang, 1998). The phylogenetic trees obtained from the PAUP analyses were used to produce a treefile for preliminary analyses in PAML using

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the one-ratio model (M0). The estimates of branch lengths produced by this model were then incorporated into the treefiles, and the tree plus branch lengths files were used as the main treefiles for all subsequent analyses in PAML (Yang, 1997).

Simulation studies indicate that the power and accuracy of the log-likelihood ratio tests used to investigate the significance of evidence for positive selection increases with the number and diversity of sequences used (Anisomova et al., 2001, 2002). These simulation studies also indicate that the tests are robust if the treelength in the analyses is greater than one substitution per codon. This criterion was met for our dataset.

The following models were used to analyze the dataset for each gene region: M0 (single rate model), M1 (neutral model), M2 (basic selection model), M7 (continuous distribution model), and M8 (continuous distribution plus selection model). Codon frequencies were estimated from the average nucleotide frequencies at the three-codon positions for all runs. Log-likelihood ratio tests were used to test for significant differences in the fit of the models incorporating selection relative to their (nested) counterparts that did not allow positive selection (Nielsen and Yang, 1998). We focused on comparing model 1 (neutral model) results to model 2 (selection) results, and model 7 (continuous distribution) to model 8 (continuous plus selection), as recommended by Yang et al. (2000). We also compared model 8 to model 8 with the value of omega fixed at one, which has been used as a stringent test in some recent analyses (e.g. Clark and Swanson, 2005). These tests provide a useful series of metrics for interpreting the significance of the results. The order of stringency of the LRTs is as follows: M7 versus M8 < M1 versus M2 < M8 versus M8 fixed omega. We used the Bayes empirical Bayes method implemented in the most recent version of PAML to estimate posterior probabilities of selection on each codon. This method allows assessment of the probabilities that specific codon sites in the gene in question are under positive selection.

To check that the method had not converged on a local maximum (leaving a global maximum undetected) we carried out several runs for each set of models, using three initial values (0.5, 2 and 10) for kappa (transition/transversion ratio) and omega (0.1, 1 and 10). The final likelihoods were compared and the highest likelihood taken as the best estimate. We did not detect any case where the initial analysis was trapped on a local maximum.

3. Results

Our analysis showed strong evidence for positive selection on the *RNASEL* gene, with an omega of 2.92 at 7% of the codon sites for the discrete approximation model (M2), and 2.32 (14% of sites) for the continuous approximation plus selection model (M8). The log-likelihood ratio tests (LRT's) were highly significant ($P < 0.01$) in every comparison (M2 vs. M1, M8 vs. M7, and M8 vs. M8 with omega fixed at one). Using the Bayes empirical Bayes method (Yang et al., 2005), we estimated significant posterior probabilities of positive selection at 10 codon sites under the continuous approximation plus selection (M8) model (the first number lists the codon, the letter denotes the common amino acid at that site, and the fraction in parentheses is the estimate of the probability that the site is under positive selection): 40R (0.96), 47K (0.95), 102Q (0.96), 178K (0.96), 302E (0.96), 322Y (0.99), 323H (0.98), 618C (1.0), 647N (0.98), 654H (0.99). A number of other sites also showed high posterior probabilities of being under positive selection under both models: posterior probabilities of positive selection higher than 75% were estimated at 35 other sites. Thus, these sites showed an elevated posterior probability of being under positive selection, relative to the random expectation (Yang et al., 2005).

One site showing polymorphism associated with prostate cancer risk (Li and Tai, 2006), Asp541Glu, also showed an elevated probability of positive selection in our analysis ($\omega = 2.19$, posterior probability = 82%). This polymorphism does not reduce the RNase L activity (Xiang et al., 2003), but has been associated with risk of prostate cancer in some studies. In a study of a Japanese population sample, homozygotes for Asp541 showed increased risk for prostate cancer (Nakazato et al., 2003). In contrast, homozygotes for Glu541 were found to be at higher risk in a European–American Caucasian sample Q2 by Noonan-Wheeler et al. (2006). Shook et al. (2007) found an elevated risk for Glu541 homozygotes in Hispanic Caucasians, but no association was found in non-Hispanic Caucasians or African Americans. Other studies have also found no association between Asp541Glu and the risk of prostate cancer (e.g. Maier et al., 2005). Hence there is variation across studies, populations and ethnic groups in the relationship between Asp541Glu and prostate cancer risk, and in the specific genotypes that confer susceptibility. A recent meta-analysis comparing multiple studies concluded that there is significant evidence across studies for an effect of polymorphism at this locus on prostate cancer (Li and Tai, 2006). However, the study also demonstrated significant heterogeneity among studies of different populations for the association of this polymorphism with both sporadic and hereditary prostate cancer risk. We note here that the associations found could be due to spurious results, such as Type I errors.

We also carried out a lineage specific analysis of positive selection to determine if there was any evidence of positive selection on the human lineage in particular. Lineage-specific analyses have reduced power relative to codon-specific analyses because they average estimation of selection across the entire gene. The ML model indicated a relatively high ratio of non-synonymous to synonymous substitutions on the *RNASEL* locus along the human lineage, with an average ω of 0.92. Although the ratio does not exceed one (and hence does not exceed the threshold for positive selection), it appears elevated relative to the average ratio of 0.51 across the rest of the tree.

4. Discussion

The molecular evolutionary analyses reported here provide evidence that positive selection has acted on the *RNASEL* locus, a gene that shows variation associated with susceptibility to

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infection by XMRV and with predisposition to prostate cancer. This inference is also supported by the results of Bustamante et al. (2005), who found highly significant evidence of positive selection ($p = 0.004$) on *RNASEL* using comparisons of human intraspecific polymorphism with human-chimp divergence in a survey of over 11,000 genes. Such positive selection is found most commonly in the context of parasite-host antagonistic coevolution, and such interactions provide some of the best evidence for positive selection via antagonistic interaction in many taxa (Hughes, 1999). For *RNASEL*, the available evidence suggests that its connection with prostate cancer may have evolved, at least in part, in the context of resistance to infection by the XMRV virus. Previous research suggesting the action of positive selection on XMRV (Urisman et al., 2006) is also consistent with this interpretation.

Immune system surveillance of incipient tumors (Dunn et al., 2004), tradeoffs between immune function and testosterone production (e.g. Verhulst et al., 1999; Muehlenbein and Bribiescas, 2005), or microbe-induced inflammatory reactions favoring the development of cancer (Clevers, 2004) all provide potential explanations for a connection between loci associated with both immunity to specific pathogens and risk of cancer. In the case of *RNASEL*, the ability of the gene to induce apoptosis, and the ability of the virus to inhibit apoptosis, may be an important locus of interaction.

Conflicting results have been found concerning the roles of specific variants in *RNASEL* in both hereditary and sporadic prostate cancer (Li and Tai, 2006). For example, the homozygous Gln462 genotype in the Arg462Gln polymorphism is associated with increased risk for sporadic (non-hereditary) cases of prostate cancer in Caucasians (Casey et al., 2002), whereas the reverse association has been found for hereditary prostate cancer in Asian populations (e.g. Nakazato et al., 2003). Li and Tai (2006) also found significant heterogeneity across all studies for the effect of Arg462Gln on hereditary prostate cancer in their meta-analysis. The same situation occurs for polymorphism associated with prostate cancer at Asp541Glu, as described in Section 3 (see above). Li and Tai (2006) argued that the effect of particular mutations might be specific to particular ethnic groups in some cases. Antagonistic coevolution can generate polymorphism in the virus for exploitation of specific host genotypes and polymorphism in the host for resistance to specific viral genotypes (Frank 2002). Hence, variation in which allele is associated with risk of prostate cancer (in association with viral infection) in different populations or ethnic groups is not unexpected in this context. Indeed, variation in patterns of positive selection among ethnic groups is common in analyses of the human HapMap data (Voight et al., 2006), and genes associated with immune function are particularly likely to show this kind of variation.

The discovery that Arg541Glu shows a high posterior probability of positive selection, and also shows an association with prostate cancer (Li and Tai, 2006), indicates that this site may have been an important locus of viral-host coevolution, and consequent risk of prostate cancer. That is, variants at this position may show variation in susceptibility to the virus, and consequent variation in the risk of prostate cancer associated with prolonged viral infection. The specific variants that confer susceptibility apparently differ among populations and ethnic groups, consistent with independent coevolutionary trajectories in different populations.

Our study also revealed a number of other sites that show high posterior probabilities of positive selection. These findings suggest that variation at these sites has been associated with antagonistic coevolution with parasites over the course of mammalian evolution. Indeed, evidence from the evolutionary theory of host–pathogen interactions indicates that natural selection will continually favor new variants that enhance parasite virulence and host resistance, and this can lead to the evolution of multiple variants at multiple sites over evolutionary time (Frank, 2002). Investigation of linkages between these selected sites, and prostate cancer risk or progression, may yield novel insights into the evolutionary-genetic basis of this disease.

Recent research suggests that other loci may also mediate susceptibility to cancer in the context of interactions between the immune system and cancer. For example, positive selection has been documented for the antimicrobial beta-defensin genes of some mammals (Maxwell et al., 2003; Luenser and Ludwig, 2005), some of which exhibit aberrant expression in prostate cancer (Donald et al., 2003), and for the alpha-defensins (Lynn et al., 2004; Patil et al., 2004), which are highly overexpressed in colon cancer (Nam et al., 2005).

Uncited references Q3

Crespi and Summers (2006), Hassel et al. (1993), Page (1996), Rokman et al. (2002), Simard et al. (2003), Walsh (2003) and Wiklund et al. (2004).

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