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Implications for RNase L in Prostate Cancer Biology

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ABSTRACT: Recently, the interferon (IFN) antiviral pathways and prostate cancer genetics and have surprisingly converged on a single-strand specific, regulated endoribonuclease. Genetics studies from several laboratories in the U.S., Finland, and Israel, support the recent identification of the RNase L gene, *RNASEL*, as a strong candidate for the long sought after hereditary prostate cancer 1 (*HPC1*) allele. Results from these studies suggest that mutations in *RNASEL* predispose men to an increased incidence of prostate cancer, which in some cases reflect more aggressive disease and/or decreased age of onset compared with non-*RNASEL* linked cases. RNase L is a uniquely regulated endoribonuclease that requires 5′-triphosphorylated, 2′,5′-linked oligoadenylates (2-5A) for its activity. The presence of both germline mutations in *RNASEL* segregating with disease within HPC-affected families and loss of heterozygosity (LOH) in tumor tissues suggest a novel role for the regulated endoribonuclease in the pathogenesis of prostate cancer. The association of mutations in *RNASEL* with prostate cancer cases further suggests a relationship between innate immunity and tumor suppression. It is proposed here that RNase L functions in counteracting prostate cancer by virtue of its ability to degrade RNA, thus initiating a cellular stress response that leads to apoptosis. This monograph reviews the biochemistry and genetics of RNase L as it relates to the pathobiology of prostate cancer and considers implications for future screening and therapy of this disease.

BIOCHEMISTRY AND BIOLOGY OF THE 2-5A/ RNASE L SYSTEM

RNase L is a fascinating regulated endoribonuclease that functions in the molecular pathways of interferon (IFN)[1] action against viral infections (*1*, *2*). In the mid-1970s, I. M. Kerr and colleagues discovered the 2-5A synthetases (2′,5′-oligoadenylate synthetase, OAS), enzymes activated by double-stranded RNA (dsRNA) that converts ATP to PPi and a series of short 2′ to 5′ linked oligoadenylates, collectively referred to as 2-5A [$p_x(A2'p)_nA$, $x = 1-3$, $n =$ 2 to ≥ 4] (Figure 1) (*3*, *4*). IFN treatment of cells induces a family of OAS genes through the JAK−STAT signal transduction pathway (*5*). To date the only well-established biochemical function of 2-5A is activation of RNase L. For instance, no 2-5A binding activity was observed in cytoplasmic extracts of several different organs of RNase $L^{-/-}$ mice (*6*). The activators of RNase L must have at least one (in humans) or two (in mice) 5′-phosphoryl groups and a minimum of three adenylyl residues in 2′ to 5′ linkages (reviewed in *7*). The significance of the dsRNA requirement for 2-5A synthetase activity is that it is a common intermedi-

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[1] Abbreviations: IFN, interferon; HPC1, hereditary prostate cancer 1 allele; 2-5A, 5′-triphosphoryl, 2′,5′-linked oligoadenylate between three and about five adenylyl residues in lengths; LOH, loss of heterozygosity; OAS, 2-5A synthetase or 2′,5′-oligoadenylate synthetase.

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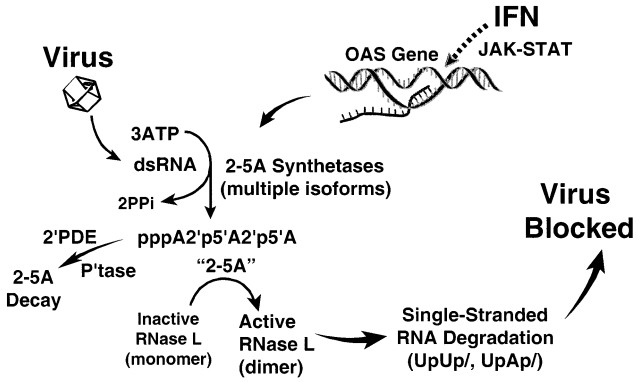

FIGURE 1: Role of the 2-5A/RNase L system in the antiviral activity of IFNs. 2′-PDE, 2′,5′-phosphodiesterase; P'tase, phosphatase.

ate or byproduct of viral infections. Thus the 2-5A synthetases may be regarded as receptors/sensors for the presence of viral dsRNA that allow the cell to respond by initiating a host-defense mechanism resulting in activation of RNase L. Accordingly, mice lacking RNase L show enhanced susceptibility to encephalomyocarditis virus (*6*).

The 2−5 Synthetase Gene Family. 2-5A synthetases catalyze the following reaction:

$$\text{nATP} \xrightarrow{\text{dsRNA}} \text{ppp5′A2′(p5′A)}_{n-1} + (n-1)\text{ppi}; \quad \text{where } (n \geq 2)$$

These enzymes, however, are much more versatile than the above reaction shows. 2-5A synthetases have broad 2′-5′-nucleotidyl transferase activity and will produce a variety of alternative products in which 2′,5′-adenylyl residues are donated to different acceptors, such as NAD or A5′p$_4$5′A (reviewed in *8*). In humans there are four related genes (OAS1, OAS2, OAS3, and OASL) encoding eight or more isoforms of 2-5A synthetase as a result of alternative splicing. 2-5A synthetases encoded by OAS1 (type I enzymes) have molecular weights of 42, 44, 46, and 48 kDa, form tetramers, and contain a single consensus sequence (*8*−*11*). OAS2 encodes the type II enzymes of 79 and 83 kDa each with two consensus sequences that form dimers. The type III enzyme (encoded by OAS3) is a 121 kDa monomeric enzyme with three consensus sequences. OASL encodes a 58 kDa protein homologous to 2-5A synthetases but which lacks 2-5A synthetase activity and contains two ubiquitin-like domains at the C-terminus (*12*). The reason for heterogeneity among different 2-5A synthetase isoforms is unknown. However, one isoform of OAS1 (*9*−*2*) is capable of mediating apoptosis through a bcl2 family homology type 3 (BH3)-domain, by interacting with bcl-2 and bclx(L) (*13*). Recently, M. Brinton and colleagues mapped the flavivirus resistance allele (flv^r) in mice to the OAS1b gene (*14*), one of nine mouse OAS1 genes (*15*, *16*). Mice with a mutation in OAS1b are susceptible to flavivirus infections, including West Nile virus (*14*, *17*, *18*). Mouse OAS1b encodes a species of 2-5A synthetase with a deletion in the P-loop motif suggested to be part of the ATP binding domain (*9*, *19*).

Properties of RNase L. RNase L, a 741 amino acid protein in humans, has an interesting arrangement of structural and functional domains (Figure 2). In the absence of 2-5A, the N-terminal half of RNase L represses the ribonuclease domain in the C-terminal region (*20*, *21*). The repressor part

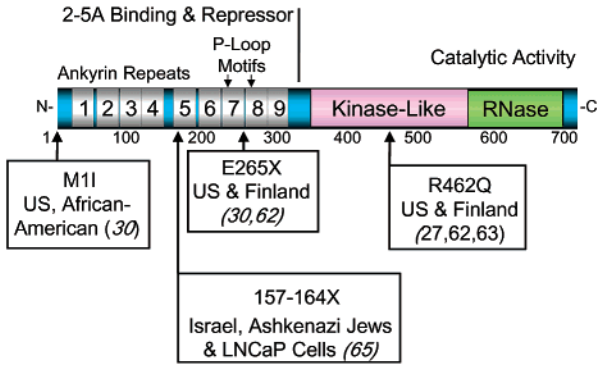

FIGURE 2: *RNASEL* mutations in different populations of prostate cancer cases aligned to the domain structure of RNase L. P-loop motifs, phosphate-binding loop motifs.

of RNase L consists of nine ankyrin repeats, a relatively common type of protein/protein interaction domain (*2*). What distinguishes the ankyrin repeats in RNase L is the presence of a duplicated P-loop like motif (GKT) in ankyrin repeats 7 and 8, the lysine residues of which are implicated in 2-5A binding (*1*). An isolated fragment of RNase L containing just the nine ankyrin repeats binds 2-5A as well as the full-length protein (*1*). The ankyrin repeat homology in RNase L also suggests regulation by interacting proteins. One candidate is the ATP-binding cassette (ABC) homology protein, RLI(HP68) that inhibits RNase L function when overexpressed and enhances RNase L activity when levels are decreased with antisense (*22*, *23*). However, these effects may be indirect because other investigators showed no physical interaction between RNase L and RLI(HP68) (*24*). There are also several protein kinase-like domains in the C-terminal half of RNase L (Figure 2). However, some conserved kinase residues are absent, and attempts to obtain kinase activity were unsuccessful (*25*). Therefore, while RNase L is predicted to be a protein kinase on the basis of sequence comparisons experimental evidence is lacking (*26*). It is difficult, however, to completely rule out the RNase L might be a kinase in vivo. Evidence does suggest that the protein kinase-like region is involved in enzyme dimerization. A missense mutation (K392R) in protein kinase-like domain II of RNase L, implicated in binding ATP in kinases, prevented RNase L dimerization and activation (*25*). Similarly, a naturally occurring missense mutation (R462Q) in the protein kinase-like region of RNase L in prostate cancer cases reduced enzymatic activity (*27*, and Y. Xiang and R. H. Silverman, 2002, unpublished results). The kinase-like and ribonuclease domains at the C-terminus of RNase L are related to the Ire1 kinases/ribonucleases that function in the unfolded protein response in organisms from yeast to humans (21, reviewed in *28*). RNase L, however, has been detected only in reptiles, birds, and mammals (of these only mouse and human RNase L sequences are currently available) (*29*). RNase L is present at constitutive basal levels in most mammalian cells, including prostate epithelial cells (*30*).

2-5A binds with high affinity to RNase L ($K_d = 4 \times 10^{-11}$ *M*), converting it from its inactive, monomeric state to a potent dimeric endoribonuclease (*31*, *32*) (Figure 3). Kinetic parameters with $C_{11}U_2C_7$ as substrate and p(A2′p)$_2$A as activator are $k_{cat} = 3.4\ s^{-1}$, $K_m = 180$ nM, and $k_{cat}/K_m = 1.9 \times 10^7\ m^{-1}\ s^{-1}$ (*33*). Interaction of 2-5A with the repressor region in RNase L relieves inhibition caused by the ankyrin

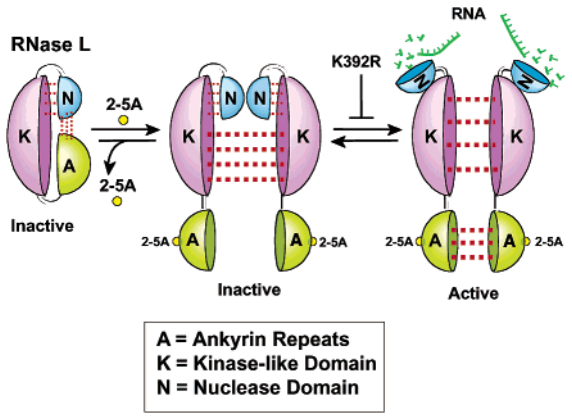

FIGURE 3: Functional model for the activation of RNase L by 2-5A. Reprinted with the permission of Cambridge University Press (*21*).

repeats, presumably as a result of inducing a conformational change in the enzyme that unmasks the dimerization and ribonuclease domains (*21*) (Figure 3). Indeed, 2-5A binding to RNase L increases dimerization affinity by a factor of 10^5-10^6 (*34*). RNase L cleaves after UpNp dinucleotide sequences (primarily UU and UA) in single-stranded RNA, but also at other sequences with lower efficiency (*35*,*36*). The 2-5A system also causes a localized activation of RNase L in the vicinity of dsRNAs activators of 2-5A synthetase (*37*). Accordingly, in EMCV-infected cells, viral RNA is preferentially degraded in comparison to cellular RNA (*38*). A truncated, recombinant RNase L lacking the ankyrin repeats showed constitutive endoribonuclease activity (i.e., no longer requiring 2-5A) (*20*). These findings suggest that a possible alternative to 2-5A activation of RNase L could be proteolysis releasing the catalytic domain. While RNase L is cleaved into N-terminal and C-terminal polypeptides in extracts of peripheral blood mononuclear cell (PBMC) from chronic fatigue syndrome (CFS) patients, in this case RNase L remains 2-5A dependent (*39*, *40*). G-actin is also degraded, and it is currently unknown if these events are involved in CFS pathogenesis (*41*).

BIOLOGY OF PROSTATE CANCER

Carcinoma of the prostate is the second leading cause of cancer deaths in men >50 years of age and the most frequent visceral cancer in males (*42*). Indeed, the American Cancer Society estimates that in the U.S. there will be 189 000 new cases and 32 000 deaths from prostate cancer in 2002 (www.cancer.org). The prostate is a walnut-sized gland of the male reproductive system located beneath the bladder and in front of the rectum that produces and stores seminal fluid (Figure 4A). The urethra runs through the prostate carrying both urine and semen. While the human prostate consists of several zones, carcinomas typically involve the peripheral glands that are often palpable during digital rectal examinations (DRE) (*42*). Precursor lesions known as prostate intraepithelial neoplasia (PIN) can progress after many years to overt carcinoma and finally to metastatic cancer (Figure 4B) (*43*). The most common sites for metastasis are regional lymph nodes and bones (pelvis and axial skeleton) (*44*). The linkage of *HPC1* to *RNASEL* suggests that RNase L directly or indirectly suppresses one of more steps in prostate tumorigenesis and/or metastasis (Figure 4B).

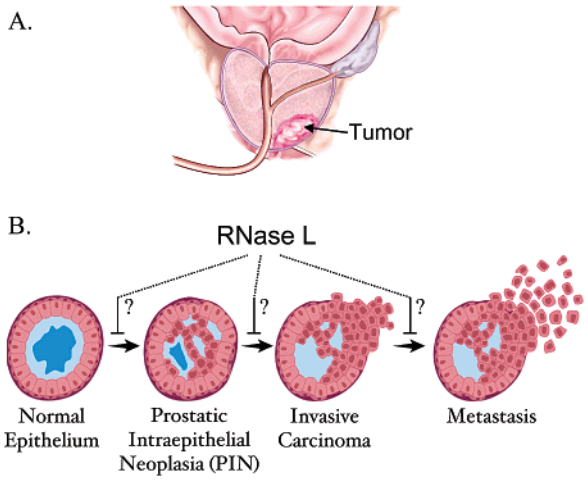

FIGURE 4: Potential role of RNase L in the suppression of prostate cancer progression. (A) Illustration of prostate with a tumor. (B) Progression of prostate cancer and steps where RNase L could interfere.

Prostate cancer is considered a disease of aging because it does not typically appear until the sixth decade (*42*). In addition to aging, hormonal, environmental, and genetic factors are all believed to play roles in the pathogenesis of prostate cancer. Androgens are implicated by the fact that prostate cancer is rare in males castrated before puberty and by the inhibition of tumor growth caused by orchiectomy or chemical hormone-ablation therapy. In addition, there is a large scientific literature on the role of androgen signaling in regulating prostate cancer (reviewed in ref *45*). Environmental causes are implicated by geographic data on prostate cancer incidence and observations that relative risk of developing prostate cancer is associated with migrations between low and high incidence regions of the world. For example, Japanese that immigrate to the U.S. experience a 4-fold increase in prostate cancer rates (*46*).

Prostate Cancer Genetics. Despite the fact that familial aggregation of prostate cancer has been appreciated since at least 1956 (*47*), it is only relatively recently that progress has been made in understanding the genetics of familial forms of the disease. Remarkably, men with three or more first-degree relatives with prostate cancer have an 11-fold increased risk compared with men that have no family history of the disease (*48*). Among populations in the U.S., African-Americans have the highest risk (*42*). Segregation analysis supports rare autosomal dominant, highly penetrant HPC gene(s) in hereditary prostate cancer with early onset (*49*). The HPC gene(s) were predicted to account for about 43% of early onset (≤55 years) disease and 9% of all cases of prostate cancer. *HPC1* was the first such prostate cancer locus, mapped in 1996 to chromosome 1q24−25 (*50*). Initial gene mapping studies placed *RNASEL* and several other genes in the critical *HPC1* region in chromosome 1q25 (*51*). Subsequently, several other loci associated with familial prostate cancer were proposed, including *HPCX* (Xq27−q28), *PCAP* (1q42), *CAPB* (1p36), *HPC20* (20q13), and *HPC2* (17p11) (reviewed in ref *52*). While one of these loci, *HPC2*, was mapped to a gene (*ELAC2*), several independent studies suggest either a minor or no role of *ELAC2* in HPC (*53*−*55*). To overcome limitations due to genetic heterogeneity and a low frequency of mutations in any particular susceptibility gene, the International Consortium for Prostate

Cancer Genetics (ICPCG) performed a joint analysis from 772 families, thus confirming linkage of hereditary prostate cancer to the *HPC1* locus (*56*, *57*). In addition, a study involving 2410 individuals, including 662 men with prostate cancer, compared several potential prostate cancer susceptibility loci (*HPC1*, PCAP, HPCX and CAPB) and demonstrated that only *HPC1*, now mapped to *RNASEL*, commonly segregated within families with the most severe cases of prostate cancer (*58*).

Genetic and Functional Studies Implicating RNase L in Prostate Cancer. RNase L was initially proposed to be a candidate tumor suppressor on the basis of its involvement in the antiproliferative activity of IFN and on the location of *RNASEL* at chromosome 1q25, a region deleted or rearranged in some breast cancers (*2*, *59*, *60*). In addition, RNase L was shown to be deficient in a human hepatoma, HEPG2 cell line (*61*). However, the first in vivo evidence implicating RNase L as a tumor suppressor was the identification of *RNASEL* as the candidate for *HPC1* (*30*). These findings were the result of a combined recombination mapping effort and candidate gene method applied to high risk U.S. families with HPC. Two of eight *HPC1* families examined contained germline inactivating mutations in *RNASEL*. In one family (065), a 795G → T substitution in exon 2 of *RNASEL* resulted in the conversion of a glutamic acid to a stop codon (E265X) in four affected brothers (three of which had clinical features associated with poor prognosis) (Figure 2). This mutation terminated translation within the 2-5A binding domain of RNase L. A similar mutation at codon 265 previously generated by in vitro mutagenesis in murine *RNASEL* eliminated 2-5A binding (*1*). The other mutation, occurring in an African-American family (097), was a methionine to isoleucine missense mutation (3G → A) in the translational start codon (Figure 2). While four of six brothers were heterozygous for M1I, all six brothers were affected with prostate cancer. Because prostate cancer is a high prevalence disease of older men, is should not be surprising that some cases would lack a germline mutation in *RNASEL*. It was suggested that the two affected brothers lacking the M1I mutation were possibly phenocopies (disease caused by other factors). This notion was supported by the fact that the two affected, nonmutation carriers in family 097 had lower tumor grades and stages than the four affected mutation carriers. Loss of the wildtype *RNASEL* allele in tumor tissue from family 065 was demonstrated both by single-strand conformation polymorphism (SSCP) analysis of microdissected tumor DNA and by immunohistochemical analysis with a monoclonal antibody against RNase L. Functional analysis of RNase L from *HPC1*-affected individuals was performed by measuring specific cleavages of rRNA after transfection of the biostable 2-5A analogue [psA-(2′ps5′A)$_3$] into lymphoblasts. M1I or E265X carriers had half the level of RNase L activity as compared with homozygous wildtype *RNASEL* family members indicating that both mutations were inactivating. The allelic frequency of E265X in the general population and non-HPC prostate cancer patients was about 0.5%, whereas M1I was only observed in the 097 HPC family (and none in 240 control and 180 non-HPC cases). In addition to E265X and M1, several missense mutations in *RNASEL* were also observed in HPC probands (G59S, 197L, 1220V, S406F, R462Q, Y529C, and D541E). Interestingly, none of the mutations or variants mapped to the ribonuclease domain of the protein (Figure 2).

A role for RNase L in HPC was further supported by a Finnish study of 116 HPC families, 492 unselected prostate cancer cases, 223 benign prostatitic hyperplasia (BPH) patients, and 566 controls (*62*). The E265X mutation was found at a significantly higher frequency (4.3%) in HPC cases than in the controls (1.8%). The frequency of E265X increased with the number of affected members per family such that families with four or more affected individuals had a frequency of E265X of 9.5% (2 of 21). There was no significant increase in the frequency of E265X in unselected prostate cancer patients compared to controls, only in HPC cases (families with three or more affected members or two affected members in which at least the index member was less than 60 years old at time of diagnosis). Complete segregation of E265X with disease was observed in only a single family, similar to prior findings in Carpten et al. (*30*). It was proposed that mutations in *HPC1* may be insufficient to always cause prostate cancer, but instead act as modifiers of disease. In support of this hypothesis, the age of onset of prostate cancer averaged 11 years earlier in E265X carriers than noncarriers in the same families. In addition, R462Q homozygotes were more frequent (22.7%) in HPC-affected patients than in controls (13.1%), but not in unselected prostate cancer cases versus controls, suggesting a role for the variant in HPC. This association also increased with the number of affected individuals per family. However, there was no association of two other missense variants, S406F and D541E, with HPC or unselected prostate cancer cases (due to complete linkage disequilibrium with E265X, frequency of G59S was the same as E265X in HPC patients compared with controls). It was concluded that while neither E265X nor R462Q alone were sufficient to account for familial clustering of the disease, both mutations in *RNASEL* affect disease onset in HPC patients.

The R462Q variant of *RNASEL* was further implicated in unselected prostate cancer cases in a study of U.S. patients (*27*). While no clearly inactivating mutations were observed in 17 familial prostate cancer patients linked to *HPC1* (average of 2.1 cases per family), R462Q and D541E variants were observed at high allelic frequencies (35% and 59%, respectively). Interestingly, the R462Q variant of RNase L had about 3-fold reduced catalytic activity in vitro, whereas another variant D541E had no affect on RNase L function. An expanded study was performed on DNA isolated from 423 unselected prostate cancer cases and 454 unaffected sibling controls. A significant association of the R462Q variant with cases was observed ($P = 0.011$). The odds ratios indicated that carrying one copy of the R462Q variant gene increased risk of prostate cancer by about 1.5-fold, while having two variant alleles doubled the risk. On the other hand, D541E, was not associated with increased risk of prostate cancer. Results implicated R462Q in up to 13% of cases, which would it make it the most prevalent genetic marker for prostate cancer (and possibly for any of the common cancers). Therefore, R462Q could be an important risk marker for prostate cancer in the general male population. Results further suggest that the decreased catalytic activity of the R462Q variant of RNase L could be responsible for the increased risk of prostate cancer. The main difference between Rokman et al. (*62*) and Casey et al. (*27*)

was that in the former study of Finnish patients, R462Q was associated only with HPC whereas in the latter study this variant predicted risk in U.S. men regardless of family history. These results suggest that familial links may be more important in some populations than others, again emphasizing the possible modifying role of RNase L in prostate cancer.

A Mayo Clinic study of U.S. patients focused on three *RNASEL* variants, I97L, R462Q, and D541E, in 438 familial cases, 499 sporadic cases, and 510 controls (*63*). While there was no association of I97L and D541E with cases, R462Q was associated with familial ($P = 0.02$) but not sporadic prostate cancer ($P = 0.92$) incidence. Curiously, however, in contrast to Rokman et al. (*62*) and Casey et al. (*27*), the Arg variant was more prevalent than the Gln variant in familial prostate cancer cases than in controls. A possible explanation for these differences is the manner in which controls were selected for these studies. For instance, in Casey et al., controls were unaffected siblings of cases whereas in Wang et al. controls were unrelated to cases (*27*, *63*). Nevertheless, in Wang et al., familial cases that were homozygous for the Gln variant were more common in node positive (15.4%) versus node-negative (7.7%) cases, in more advance stage (T3/*T*4; 11.2%) versus lower stage (T1/*T*2; 6.3%) tumors, and in high-grade (>7; 11.9%) versus low-grade (≤6; 6.5%) cases (*63*). Therefore, R462Q was associated with more aggressive disease. No E265X mutations were detected in this study, although several would have been expected based on frequencies of this mutation in the general U.S. population reported by Carpten et al. (*30*). Thus, in three separate studies, R462Q predicted risk of prostate cancer in Finnish HPC families (*62*) and in unselected U.S. prostate cases (*27*) or was associated with more advanced disease (*63*). It is somewhat surprising, perhaps, that only a 3-fold reduction in enzyme activity could have a significant impact on pathogenesis of prostate cancer. However, relatively small allelic variations in gene expression have been described that could have an impact on disease susceptibility (*64*).

An additional mutation in *RNASEL*, 471ΔAAAG, causing a frame shift at codon 157 and a translation stop after 7 additional codons, was discovered by Rennert et al. in Ashkenazi Jews at a relatively high frequency (4%) and in the model human prostate cancer cell line, LNCaP (*65*). The frequency of the mutation was higher among prostate cancer patients (6.9%) than in elderly (unaffected) men (2.4%) of this population group. The mutation was not found in 134 non-Ashkenazi prostate cancer patients or controls. Two brothers with prostate cancer were described, one was heterozygous in *RNASEL* and showed loss of the wildtype allele in tumor tissue. The other brother was homozygous for the mutation, and therefore completely lacked RNase L. Therefore, in humans as in mice *RNASEL* is a nonessential gene (*6*). On the basis of conserved, closely linked markers flanking *RNASEL*, it was suggested that the 471Δ*A*AAG mutation is a founder mutation. Rennert et al. also concluded that there is an association between this mutation in *RNASEL* and prostate cancer in Ashkenazi men (*65*). It remains to be seen if mutated *RNASEL* predisposes to any other forms of cancer. Curiously, however, RNase L levels are actually elevated in colorectal tumors (*66*).

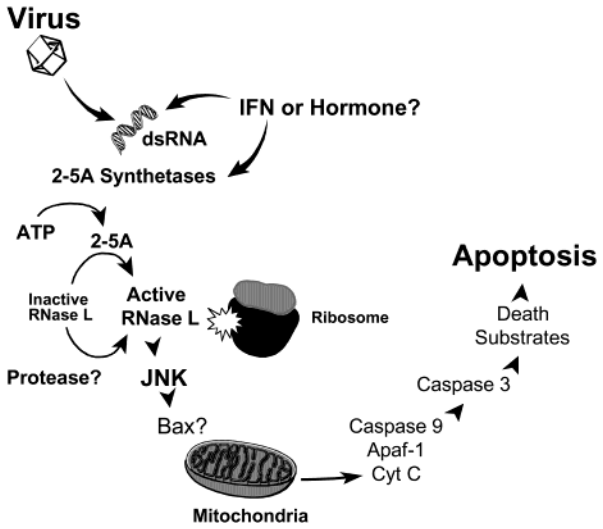

FIGURE 5: Molecular pathways for the antiviral and antitumor activities of the 2-5A/RNase L system. Cyt C, cytochrome C.

TUMOR SUPPRESSOR ROLE OF RNASE L

It has been pointed out that RNase L could tip the balance between hormonally regulated growth and cell death in the prostate (*30*). The antiproliferative and apoptotic activities of RNase L are consistent with its tumor suppressor function. Indeed, the 2-5A/RNase L pathway is implicated in mediating apoptosis in response to viral infections and to several types of external stimuli (*6*, *67*−*73*). In individuals with wild type RNase L, induction of OAS gene expression through either changing hormone status or IFN could potentially prime the pathway for activation (Figure 5). Hormonal control of OAS gene expression has been shown in a few studies, although not in prostate. For example, hydrocortisone has been reported to induce 2-5A synthetase expression in human lymphoblastoid cells (*74*). In addition, withdrawal from estrogen stimulation induces 2-5A synthetase expression and causes rRNA degradation during regression of the chick oviduct (*75*, *76*). In this regard, it will be interesting to determine if RNase L is involved in hormonally regulated cancers in women, such as breast cancer.

A viral etiology is suggested by the presence in prostate cancer patients of mutations in *RNASEL*, an established antiviral gene. Alternative evidence in support of an infectious agent includes the presence of mutations in the macrophage scavenger receptor 1 (MSR1 or SR-A) gene at chromosome 8p22 associated with increased prostate cancer risk (*77*). Interestingly, $MSR1^{-/-}$ mice have enhanced susceptibility to bacterial (*Listeria monocytogenes* and *Staphylococcus aureus*) and viral (herpesvirus type-1) pathogens (*78*, *79*). In addition, a large case-control study showed an association between prostate cancer frequency and a history of sexually transmitted diseases (*80*). Although human papillomavirus (HPV) has been associated with prostate tumors in some studies, others found no such association (reviewed in ref *81*). Nevertheless, the prostate has been recently reported to be a resident organ for mutiple viral infections, including the human polyoma JC virus and HPV (*82*).

While involvement of a viral pathogen in prostate cancer remains a possibility, novel mechanisms that have not been observed in the context of virus-infected cells are also

possible. For example, a delay in rejection of skin allografts in RNase $L^{-/-}$ mice suggests a role for RNase L in cellular immunity that could possibly contribute to tumor rejection through the immune system (*83*). In addition, alternatives to virus-generated dsRNA exist for activation of the 2-5A synthetases (Figure 5). The human brain contains long hairpins in the untranslated regions of some mRNAs that serve as substrates for adenosine deaminases that act on RNA (ADARs) (*84*). In addition, molecular evolution (SELEX) methods resulted in single-stranded and double-stranded RNA aptamers capable of activating 2-5A synthetase (*85*). If present in the prostate, similar types of RNA structures could potentially activate 2-5A synthetases. Alternately, cleavage of the repressor domain from RNase L by a protease could possibly lead to unregulated ribonuclease activity and apoptosis. Therefore, there are several possible avenues for RNase L activation in prostate cells (Figure 5).

How might RNase L activation lead to apoptosis? The degradation of 28S and 18S rRNA by RNase L in intact ribosomes has been long known as a hallmark of IFN and viral infections (*86*, *87*) (Figure 5). Cleavage of 28S rRNA by RNase L maps to the L1 protuberance implicated in formation of the exit or E site of the ribosome, possibly interfering with release of deacylated tRNA (*88*). By comparison, the fungal ribonuclease, α-sarcin, and the RNA modifying enzyme, ricin A chain, and UV light each lead to ribotoxic stress responses involving damage to the 3′-end of the large ribosomal RNA. These treatments activate the stress-activate protein kinases, c-jun NH2-terminal kinases (JNKs) (*89*, *90*). JNK activation in response to UV irradiation has been linked to apoptosis through Bax (*91*). Interestingly, phosphorylation of JNK in response to dsRNA is deficient in RNase L-null cells (*88*). Apoptosis initiated by RNase L activation requires caspase 3 activity and is characterized by the appearance of cytosolic cytochrome *c* indicating involvement of mitochondria (*73*). Both the antiviral and tumor suppressor activities of RNase L in vivo could be due to its pro-apoptotic activity, limiting viral spread or tumor growth within an organism. It would be interesting to know whether the frequency and severity of viral infection is increased in individuals with mutant *RNASEL*.

The occurrence of mutations in *RNASEL* in prostate cancer cases has implications for pathogenesis of the disease, for assessing risk, and possibly also for novel therapeutic options. For example, these findings could lead to effective methods for monitoring germline mutations in RNase L that predispose to aggressive or early onset prostate cancer. Situations where it might be useful to know the status of *RNASEL* include selecting individuals for chemoprevention trials and deciding the aggressiveness and type of therapy for prostate cancer (*92*). Identification of *RNASEL* as a tumor suppressor could provide a unique opportunity to control prostate cancer, and possible other cancers, at the level of RNA turnover. Inducing RNA damage as a means of controlling tumor growth is not a new idea. The ribonuclease, onconase, the N-glucosidase ricin A chain that attacks ribosomal RNA, and the anti-FLT-1 (VEGR receptor) ribozyme, angiozyme, have been explored as cancer therapeutics in clinical trials with varying success (*93*−*95*). An RNase L-based approach might have certain advantages in the treatment of cancers. RNase L is a candidate tumor suppressor that is normally dormant but whose antitumor cell activity can be activated by a small molecule, 2-5A (*72*, *73*). It is also possible to target RNase L to particular cancer associated RNAs, such as telomerase RNA, by linking 2-5A to antisense (*96*, *97*). In cancers where RNase L is present, including many prostate tumors, its activation by a 2-5A analogue might produce an antitumor response as was demonstrated in a mouse model of human prostate cancer (*98*). To minimize adverse effects, directing 2-5A to the appropriate cell type or to a desired RNA species could provide specificity (*99*). RNase L has long been appreciated for its unique mode of regulation and its involvement in the mechanism of IFN action. Studies on prostate cancer genetics and pathogenesis are providing a new direction for future studies on this intriguing, regulated nuclease.

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