Regulation of snoRNAs in Cancer: Close Encounters with Interferon

Shreeram C. Nallar and Dhananjaya V. Kalvakolanu

The interferon (IFN) family of cytokines regulates many cellular processes, such as transcription, translation, post-translational modifications, and protein degradation. IFNs induce growth inhibition and/or cell death, depending on the cell type, by employing different proteins. This review describes a novel growth-suppressive pathway employed by IFNs that affects rRNA levels. Maturation of rRNA involves numerous noncoding small regulatory RNA-guided processes. These regulatory RNAs, called small nucleolar RNA (snoRNAs), function as a ribonucleoprotein particle (RNP) in the nucleolus. The biogenesis of snoRNPs is dependent on core protein and assembly factors. Our laboratory recently isolated a growth-suppressive protein gene associated with retinoid-IFN-induced mortality (GRIM)-1 using a genetic screen. IFN-inducible GRIM-1 (SHQ1) is an assembly factor that controls one arm of the snoRNP machinery. GRIM-1 inhibits sno/scaRNP formation to induce growth suppression via reduction in mature rRNA levels. Loss of GRIM-1 observed in certain cancers implicates it to be a novel tumor suppressor. Certain snoRNAs have been reported to act as either oncogenes or tumor suppressors *in vitro*. Recent studies have shown that certain sno/scaRNAs are further processed into micro RNA-like molecules to control translation of protein-coding RNAs. We present a model as to how these small regulatory RNAs influence cell growth and a potential role for GRIM-1 in this process.

Introduction

THE INTERFERON (IFN) family of cytokines control a number of cellular processes, including transcription, RNA stability, RNA editing, mRNA export, translation, and protein stability, through the induction of cellular IFN-stimulated gene (ISG) products. All or some of these processes are ultimately linked to innate host defenses, such as growth suppression, pathogen elimination, autophagy, and apoptosis, while some of the ISGs extend into activating acquired immune responses and other physiological processes (Dunn and others 2006). The best example of an immediate IFN-stimulated effect is inhibition of cap-dependent translation initiation by protein kinase-R (PKR). PKR, by directly phosphorylating the eukaryotic translation initiation factor 2 α , shuts down translation of viral and certain cellular RNAs to exert a cyto-protective effect (Samuel 1979). PKR has been recently shown to influence learning and memory through GABA receptor kinetics in the central nervous system, although the mechanisms are unclear (Zhu and others 2011). The IFN-stimulated synthesis of 2'-5' oligoadenylates activates the latent Ribonuclease-L (RNaseL), which cleaves viral and certain cellular mRNAs (Zhou and others 1993). Mutations in the *RNASEL* gene, found in certain human prostate cancers, appear to diminish its enzymatic activity,

thus compromising its cyto-protective effects (Li and Tai 2006). The IFN-inducible nucleic acid-editing enzymes, ADAR1 and AID, are also involved in host defense mechanisms. Other IFN-induced gene products that participate in RNA export, Nup98 and Nup96, override the VSV-M protein-induced blockade of nuclear pore complex to allow host gene expression (Enninga and others 2002). The ISG56 protein interferes with mRNA translation by inhibiting eIF3 activity (Guo and others 2000). Certain members of the ISG56 family also promote apoptosis and inhibit papillomavirus replication. Another IFN-responsive protein, the death-associated protein kinase-1 (DAPK1), which plays important roles in regulating cell growth and autophagy, has been implicated in the regulation of excitotoxicity through the NMDA receptors and causes neuronal death (Tu and others 2010). DAPK1 has also been implicated in the translational repression of certain inflammation-associated mRNAs by directly phosphorylating the L13a subunit of ribosome (Mukhopadhyay and others 2008).

All life forms utilize modified nucleotides to regulate their nucleic acids. In prokaryotes, methylated nucleobases (^mN) in DNA by the host restriction-modification system protects their genetic material, while destroying the genetic material of the invading phage (Marinus and Morris 1973; Wilson 1991). A eukaryotic parallel of this phenomenon observed in

Department of Microbiology & Immunology, Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, Maryland.

human–mouse cell hybrids (Ruddle and Creagan 1975) helped in the initial mapping of IFN genes in humans (Creagan and others 1975). A role of this modification in eukaryotic mRNA have been reported recently (Bodi and others 2012; Dominissini and others 2012; Meyer and others 2012); in general, mN appears to confer protection for nucleic acids. In mammals, DNA methylation is associated with genomic imprinting and epigenetic phenomena often resulting in inhibition of transcription. In addition to DNA base modification, RNA molecules harbor other uniquely modified residues. It is interesting to note that certain RNA modifications show a high degree of conservation at certain positions in an rRNA species (Ofengand and Bakin 1997); and other newer modifications have been acquired depending on organismal complexity. A typical eukaryotic transcript pool consists of protein-coding RNAs and noncoding RNAs; the latter comprising ~95% of the bulk. The non-coding RNA pool is further categorized into functional classes as follows: rRNA, tRNA, snRNA, small nucleolar RNA (snoRNA), small Cajal body RNA (scaRNA), TERC, micro RNA (miRNA), and other emerging noncoding RNAs (such as eRNA, gRNA, piRNA, saRNA, and tiRNA). A balance among these RNAs is maintained in cells at a steady state that gets disturbed under conditions like stress, inflammation, cancer, and other diseases. The 4 rRNA species (28S, 18S, 5.8S, and 5S) form the main structural scaffold of a ribosome onto which ribosomal proteins are loaded forming the mature particle that translates mRNAs in the cytoplasm. The entire maturation of rRNA takes place in the nucleolus aided by myriad of factors (~200) that are not part of the mature ribosome (Kiss and others 2006; Terns and Terns 2006). Three major types of modified residues (mN , N_m , Ψ) are found in the mature rRNA. It is believed that these modifications are necessary for rRNA maturation and in some way helps ribosome assembly and function (Decatur

and Fournier 2002). Of these, N_m and Ψ are sequence-guided processes; while the basis for mN remains obscure, even though the modifying methylase has been identified. Gross inhibition of these modifications has a detrimental effect on cells (Decatur and Fournier 2003). However, the actual role(s) of these modifications are still unclear largely owing to a lack of specific and/or direct assays. The focus of this review will be on how rRNA maturation is affected by IFNs, with a specific discussion on how sno/scaRNAs influence the cellular function in conjunction with their associated protein machinery. We also describe a RNA regulatory mechanism targeted by IFNs through a novel gene product called, *GRIM1* (*SHQ1*), isolated as a growth-suppressive gene product in a genetic screen employed by our laboratory (Hofmann and others 2010). The yeast orthologue of *GRIM1*, *Shq1*, is necessary for the biogenesis of H/ACA RNAs and rRNA maturation (Yang and others 2002).

A Preview on snoRNAs and snoRNPs

Among the several noncoding RNAs that control cell growth are the snoRNAs represented by 2 structural classes *viz.*, C/D and H/ACA RNAs. More than 200 snoRNAs have been identified in mammals with a wide range in size (60–300 nucleotides). All snoRNAs are derived from RNAPol-II activity with a majority processed from intron(s) of certain protein-coding and noncoding transcripts, while some are transcribed independently. A few examples are listed in Table 1 to show the diversity of their role in rRNA maturation along with their location in the host gene. A comprehensive list of species-wise snoRNAs and their targets can be found at <http://snoopy.med.miyazaki-u.ac.jp>. H/ACA RNAs form hairpin (stem-bulge-stem-loop) or hairpin-hinge-hairpin structures, while the C/D RNAs adopt stem-loop structures that allow them to associate with specific cellular

TABLE 1. A SAMPLE LIST OF SNO/SCARNA-GUIDED PROCESSES ON RIBOSOMAL AND SPLICEOSOMAL RNA

snoRNA ID	Host Locus	snoRNA origin	Function(s)
SNORD3 (U3)	SNORD3	Independent transcription	Transcription of 45S rRNA
SNORA73 (E1/U17)	RCC1	Independent transcription	Maturation of 18S rRNA
SNORD118 (U8)	SNORD118	Intronic	Maturation of 18S rRNA
SNORD14	RPS13	Independent transcription	Maturation of 5.8 and 28S rRNAs
SNORD22	SNHG1	Intronic	Maturation of 18S rRNA
SNORA21	RPL23	Intronic	Maturation of 18S rRNA
SNORD54 (U54)	RPS20	Intronic	Ψ of U _{4401/4470} in 28S rRNA
SNORD35A	RPL13A	Intronic	N_m of G ₆₄₄ in 18S rRNA
SNORD35B	RPS11	Intronic	N_m of C ₄₅₀₆ in 28S rRNA
SNORA70	RPL10	Intronic	Ψ of U ₁₆₉₂ in 18S rRNA
SNORA65	RPL12	Intronic	Ψ of U _{4373/4427} in 28S rRNA
SNORD68	RPL13	Intronic	N_m of U ₄₂₈ in 18S rRNA
SNORA69	RPL39	Intronic	N_m of A ₂₃₈₈ in 28S rRNA
SNORA74 (U19)	MATR3	Intronic	Ψ of U ₆₉ 5.8S rRNA
SCARNA8 (U92)	FAM29A	Intronic	Ψ of U ₃₆ 18S rRNA
SCARNA13 (U93)	SNHG10	Intronic	Ψ of U _{3741/3743} in 28S rRNA
			Ψ of U ₈ in SNORD3 (U3)
			Ψ of U _{34/44} in RNU2 (snRNA-U2)
			Ψ of U ₅₄ in RNU2 (snRNA-U2)
			Ψ of U ₅₃ in RNU5 (snRNA-U5)

snoRNA, small nucleolar RNA; scaRNA, small Cajal body RNA.

FIG. 1. A planar representation of H/ACA RNA. These RNAs can have single or double hairpin structures. The central bulge (Ψ pocket) hybridizes to complementary sequences in rRNA (in blue) and the unpaired base (in red) is the uridine targeted for Ψ by DKC1. The specific example shown is SNORA65 hybridizing to 28S rRNA [redrawn from (Wu and Feigon 2007)]. Structure can be accessed from NCBI site using PDB ID code 2P89. Color images available online at www.liebertpub.com/jir

proteins to form a functional snoRNP. A planar representation of snoRNAs is shown in Fig. 1.

Formation of a snoRNP

A tertiary structural feature in the intronic portion of the hnRNA is believed to be responsible for loading the protein(s) that specifically recognize the snoRNA region. There are some differences between C/D and H/ACA RNAs during the ribonucleoprotein particle (RNP) assembly. After the intronic region has been spliced completely, other factors (exonucleases) trim the flanking intronic sequence, thus leaving the snoRNA region intact with its bound protein complex. The protein complex for nascent and functional C/D RNP consist of Fibrillarin (FBL, an RNA methylase), Nop56, Nop58, and the kink-turn motif is recognized by NHP2L1 (NHPX, 15.5-kDa protein) (Tyc and Steitz 1989). In H/ACA RNA, the apical loop and upper stem region are recognized by NHP2 and the nascent RNP consists of Dyskerin (DKC1, the pseudouridylyase), NOP10, and NAF1; the premature H/ACA RNP is devoid of NAF1 and GAR1 is incorporated to form the functional H/ACA RNP (Balakin and others 1996). The above processes occur in the nucleoplasm. Using pure components (protein and RNA), it is easier to assemble an archeal snoRNP *in vitro*, while it has been challenging to assemble a mammalian snoRNP.

Factors involved in snoRNP maturation

In addition to the proteins present in the mature snoRNP, other structural (Nopp140 (Isaac and others 1998), SMN (Pellizzoni and others 2001) etc.), enzymatic (RUVBL1, RUVBL2 (King and others 2001) etc.), transport (CRM1, PHAX (Boulon and others 2004) etc.) proteins are also needed for snoRNP assembly and probably maturation, although they are spatially and temporally separated from the site of snoRNA transcription and initial snoRNP formation. In addition, some assembly factors (SHQ1, NAF1, HSP90, BCD1 etc.) are needed for nascent snoRNP formation, but are not found in the later stages.

Function of snoRNPs

Transcription, processing, and maturation of rRNA occurs in the nucleolus with the aid of numerous snoRNPs, ribosomal proteins, and other factors. In addition, 5S rRNA (RNAPol-III transcribed and processed outside the nucleolus) is imported into the nucleolus to be assembled into the ribosome (Zhang and others 2007). After mature snoRNPs have formed, they transiently accumulate in Cajal bodies

(CBs) before moving to the nucleolus, where they assist in the formation, processing, and maturation of rRNA. In CBs, C/D RNPs reside longer than H/ACA RNPs and hence, it is believed that some additional processing or maturation events occur in this organelle that remain to be characterized (Richard and others 2003). Once they have been delivered to the nucleolus, they assist either in RNAPol-I transcription or processing of the rRNA primary transcript (i.e., generation of mature 28S, 18S, and 5.8S rRNA molecules) or in rRNA folding by either of the 2 modifications (N_m or Ψ). Table 1 summarizes the function of few snoRNAs. Processing, modifications, and folding of rRNA are simultaneous events and so assigning a specific role to a particular site modification has not been greatly successful. Nonetheless, simultaneous blocking of multiple modifications delays or hinders rRNA maturation kinetics leading to imbalanced ribosomal subunits (60S and 40S) in the cytoplasm.

Regulation of snoRNAs in Cancer

Most of the snoRNA/RNP function has been deciphered using genetic studies of budding yeast; 5 snoRNAs considered crucial for cell viability (see Table 1) are involved in rRNA processing and not in modification of rRNA. Similarly, deletion or depletion of snoRNP proteins and their accessory factors manifests as synthetic lethality or poor growth of yeast cells, respectively (see ref (Fromont-Racine and others 2003), for a comprehensive list). In mammalian cells, RNAi-mediated depletion of snoRNP proteins also results in loss of snoRNA levels (Grozdanov and others 2009). On the contrary, cancer cells have higher levels of rRNA synthesis and protein turnover compared to their normal cells implying that the entire ribosome biogenesis pathway operates at very high rates (Belin and others 2009; Silvera and others 2010). The usefulness of some snoRNAs as prognostic indicators has also been evaluated in cancer patients using unbiased screens. However, recent studies have found that not all snoRNAs follow a similar trend in cancers and some of the protein factors are in fact downregulated. Studies performed in cell lines by ectopic expression of snoRNAs have indicated that they can act as either a tumor suppressor or an oncogene. Nonetheless, the actual biological role of a snoRNA-guided modification remains to be elucidated for developing novel therapeutics.

In some cancers, certain snoRNAs are present in high levels compared to others in the same group (C/D or H/ACA RNA). The location of such snoRNAs in frequently amplified chromosomal regions appears to be the primary cause for these observations. Forced expression of these

snoRNAs (*SNORD33*, *SNORD44*, *SNORD66*, *SNORD76*, and *SNORA42*) in cancer cell lines is growth beneficial, while depletion retards cell growth and colony formation (Liao others 2010; Appaiah and others 2011; Mei and others 2011). Hence, these can be called oncogenic snoRNAs. An opposite trend is seen with certain other snoRNAs that are present in very low levels or the entire locus is lost due to chromosomal breakage. The expression of these snoRNAs (*SNORD50*, *SNORD43*, *SNORD44*, and *SNORD48*) appears to inhibit cancer cell growth *in vitro* similar to tumor suppressors (Dong and others 2008, 2009; Gee and others 2011). However, genetic deletion of homologous snoRNA in yeast is not deleterious to cell function, that is, no phenotypic score. Although yeast offers a suitable model for genetic analyses, it is a poor choice for querying the relevance of snoRNA-guided modifications in cellular function and growth as many mammalian sno/scaRNAs are absent in the yeast. Thus, these snoRNAs appear to influence cell characteristics outside their canonical pathway in cancer cells, which remains to be established. Using bioinformatics, some snoRNAs have been identified to undergo further processing and act as miRNAs (called sno-miRs) (Scott and others 2009). This area of snoRNA research is emerging as demonstrated by the presence of snoRNA-derived sequences in Ago1/2 complexes, the core proteins of RISC (Ender and others 2008). Considering that the levels of such sno-miRs are very low in a cell, deciphering the functional target(s) of such sno-miRs will be addressed in the coming years.

Among sno/scaRNAs, the role of *TERC* is so far one of the widely studied; it is needed for all epithelial-derived cancers to protect their aneuploid chromosomal sets. *TERC* is a special sno/scaRNP that does not function in the nucleolus; *TERC* helps TERT to synthesize the telomeric repeat sequence (Collins and Mitchell 2002). Mechanistic details of how *TERC* is transferred to TERT are unclear at present. Polymorphisms found in the *TERC* locus are associated with a few malignancies (Vulliamy and Dokal 2008). Even in the absence of a functional TERT, an alternate pathway (Bryan and others 1997), as seen in sarcomas, can maintain chromosomal ends. Moreover, chemically induced cancers have been reported in a *Terc*^{-/-} mouse model and fifth and later generations of *Terc*^{-/-} mice produce highly metastatic squamous cell carcinoma (Rudolph and others 1999; Bojovic and Crowe 2011). In humans, similar incidence of squamous cell carcinoma have been reported in patients with the X-linked form of dyskeratosis congenita (DC), which is due to point mutation(s) in the *DKC1* gene that reduces *TERC* levels. DC presents clinically as a multisystem anomaly with features of bone marrow failure, abnormal skin pigmentation, leukoplakia, and increased susceptibility to cancer (Kirwan and Dokal 2008). However, other snoRNAs are not affected to the extent of *TERC* by the mutant *DKC1* protein(s) although the biochemical mechanisms are unknown. A point mutation in the *DKC1* gene (S⁴⁸⁵G) identified in pituitary adenoma (Bellodi and others 2010b) does not affect *TERC* levels in spite of being a less active protein. Interestingly, none of the point mutations identified so far in DC are in the catalytic region of the protein. A *DKC1* isoform lacking the C-terminal 94 amino acids localizes in the cytoplasm, promotes growth, and cell adherence appears to be independent of its known function in the nucleolus (Angrisani and others 2011). Two H/ACA RNAs (*SNORA36* and *SNORA56*) are

produced during *DKC1* splicing. However, the variant transcript that codes for the novel isoform does not produce *SNORA56*, which is the precursor for miRNA. Autosomal dominant and recessive forms of DC have been associated with point mutations in *TERC*/*TERT*/*TINF2* and *NHP2*/*NOP10*, respectively (Vulliamy and Dokal 2008). Thus, in all cases mentioned above, critically shortened telomeres elicit genomic instability that finally resolves as cancer. Hypomorphic *Dkc1* mutant mice present clinical features of DC from the first generation pointing to a telomere/telomerase-independent pathway of genomic instability with high incidence of spontaneous tumors (Ruggero and others 2003). Cancers of the breast, lung, and B-cell lymphomas were frequent in this genetic model. Interestingly, lymphocytes express high levels of the cytosolic *DKC1* isoform (Angrisani and others 2011). On the contrary, expression of TERT immortalizes primary cell lines (Roy and others 2004) and malignant tumors develop in transgenic mouse models of E6 (from high-risk HPV16) expression in the skin (Song and others 1999). In the presence of E6 oncoprotein, the substrate specificity of E6AP (UBE3A; an E3 ubiquitin ligase) is altered leading to proteosomal degradation of ubiquitinated NFX1 (Gewin and others 2004) and p53 (Scheffner and others 1990) proteins; the former is a transcriptional repressor of *TERT*, while the latter is a cell cycle regulator. Thus, this mechanism actively promotes cell cycle and helps telomere length maintenance. In addition, translation of p53 mRNA is impaired under stress when *DKC1* levels are low, thus mitigating the normal DNA checkpoint response (Montanaro and others 2010). This may explain to some extent why hypomorphic *Dkc1* mouse embryonic fibroblasts are resistant to p53-mediated senescence (Bellodi and others 2010a). However, p53-dependent responses appear normal and only cell proliferation is severely affected in *Dkc1*-null liver cells that could survive for months without adverse effects (Ge and others 2010) and did not undergo immediate apoptosis as seen in *Dkc1*^{-/-} embryos during development (He and others 2002).

While integration of HPV DNA is a stochastic process with no obvious preference to any genomic loci, adeno-associated viruses (AAV) prefer to integrate at a specific site, which happens to be an imprinted locus. Studies in mouse models have uncovered high-frequency integration of AAV on chromosome 12 near the *Meg3* locus (Donsante and others 2007). This region is rich in snoRNA and miRNA genes and the integration event potentially affecting host gene expression has been implicated in the development of hepatocellular carcinoma. The syntenic region in humans is proposed to contain tumor suppressors. Expression of the noncoding *MEG3* transcript is severely suppressed in various tumor types and tumor cell lines, while genomic deletion was associated with aggressive tumors (Zhou and others 2012). *Meg3* RNA upregulates p53 levels (Zhou and others 2007), by downregulating *Mdm2* levels, and promotes angiogenesis by increasing *Vegfa* and *Vegfr1* levels (Gordon and others 2010). The adjacent *Meg8* (*Rian*) locus produces a long non-coding RNA that is differentially expressed in developing embryonic tissues (Gu and others 2012). Silencing of *Meg8* expression is needed for induced pluripotent stem cell growth (Stadtfeld and others 2010) akin to cancer cell growth. In spite of a lack of sequence conservation between human and mouse in and around *MEG3*-*MEG8* loci, numerous snoRNAs and miRNAs are produced in this region in both species. Multiple copies of *SNORD113* and *SNORD114* are

present only in humans, while numerous miRNAs are present in both human and mouse in this chromosomal locus. The function(s) of *SNORD113* and *SNORD114* are not known at present.

Cell type-specific imprinting regulates the *Snurf-Snrpn-Ube3a* locus in adult tissues. This locus contains multiple copies of *SNORD115* and *SNORD116* whose nascent RNAs have C/D RNA features with no known role on rRNA processing. Clinically, loss of the maternal allele manifests as the Angelman syndrome, while the Prader–Willi syndrome results due to loss of the paternal allele (Nicholls and Knepper 2001); autism-spectrum disorders are seen in both cases. Studies have shown that *SNORD115* is processed to a smaller RNA to be incorporated into hnRNPs and assists in splicing of hnRNA to generate transcript variants and, hence, proteome diversity (Kishore and others 2010). *SNORD115* is needed for the correct splicing of *Htr2c* (a serotonin receptor) RNA to generate a receptor with optimal ligand binding and signaling characteristics (Kishore and Stamm 2006). Studies have implicated loss of *SNORD115* (de los Santos and others 2000) or *SNORD116* in the Prader–Willi syndrome (Runte and others 2005; Sahoo and others 2008). Other studies in mouse models have indicated *Ube3a* as the candidate gene and excluded *SNORD115/116* to be the cause (Jiang and others 1998). Additionally, *Ube3a* undergoes alternative splicing, while *HTR2C* mRNA is edited by ADAR2 to generate receptor diversity (Flomen and others 2004). Since, ubiquitin ligases can modulate the strength and duration of the signal by regulating ligand/receptor levels, both UBE3A isoforms and *SNORD115/116* may contribute to some extent in these neurological conditions, and recently this locus has been implicated in pancreatic endocrine insufficiency (Stefan and others 2011).

Another widely studied alternatively spliced gene is *PKM* (Noguchi and others 1986). The M2 transcript variant is the dominant form observed in embryonic tissues, normal proliferating cells, and cancer cells (Mazurek and others 2005). Spontaneously immortalized 3Y1 fibroblasts show transcripts only for the M2 variant, while M1 variant is undetectable (Nallar and Kalvakolanu, unpublished). Transcription factors (ZFX, MYC, E2F1, STAT3 etc.) have been implicated in upregulating hnRNPs A1/A2 and PTB to suppress M1 transcript variant in cancer cell lines (Clower and others 2010) (see (Chaneton and Gottlieb 2012) for a review). What determines alternate splicing of *PKM* RNA in a physiological context is not known at present; however, a snoRNA-derived regulatory RNAs may be indirectly involved. It is believed that the M2 isoform helps partitioning carbon pool to sustain accelerated growth and at the same time helps cell survival when oxidative stress levels are high. The C/D snoRNAs (*SNORD32A*, *SNORD33*, and *SNORD35A*) produced from *RPL13A* introns have been suggested to regulate oxidative stress during carcinogenesis (Michel and others 2011), while *RPL13A* regulates translation of certain cellular mRNAs under specific conditions (Mukhopadhyay and others 2008). Interestingly, hnRNPA1 is also involved in alternate splicing of *SMN2* mRNA, by antagonizing hnRNPQ1, producing a defective SMN2 protein leading to spinal muscular ataxia in a *Smn1*^{-/-} mouse background (Chen and others 2008). SMN proteins are needed for maturation of snoRNPs and the exact mechanism for this tissue-specific defect is unclear. Regulation of alternate splicing by snRNA-U2 harboring noncanonical Ψ may be relevant in such scenarios (Wu and others 2011).

Recent studies indicate AID regulates certain non-IgG loci rich in repetitive sequences, such as *SNHG3* or *RCC1* locus (Kato and others 2012). The E1 snoRNA (U17 or *SNORA73A/B*) produced from this locus (Pelczar and Filipowicz 1998) undergoes uridine insertional editing. Earlier biochemical analyses had shown that E1 snoRNPs were asymmetric; multiple copies of E1 snoRNAs were present in the snoRNP along with other noncore proteins (Eliceiri 2006). The most important role of E1 snoRNP is in rRNA processing to generate the mature 18S rRNA (Mishra and Eliceiri 1997).

The spliceosome is a multicomponent RNP and all 5 snRNAs (RNUs) carry modified residues (N_m and Ψ) guided by 19 scaRNAs. Fragments identical to *SCARNA3*, *SCARNA8*, *SCARNA15*, and *SCARNA16* have been isolated from Ago1/2 protein complexes and reported to function like miRNAs (Ender and others 2008). *SCARNA15*-derived miRNA-like fragment targets the Mediator subunit *CDK11* mRNA, thus impacting transcription. In the same study, fragments identical to *SNORA3*, *SNORA36B*, *SNORA50*, and *SNORA56* were also isolated. The host genes for most of these snoRNAs have a direct role in translation. Still it is unclear whether processing of these snoRNAs into miRNAs is a default cellular process. The consequence(s) of blocking such a process also needs to be addressed in the coming years. Nonetheless, it is reasonable to believe that this is used as a relay signal between the transcriptional and translational apparatus to maintain intracellular homeostasis.

Two other ncRNAs (*ZFAS1* and *GAS5*) hosting snoRNA genes have been implicated in the development of breast cancer. *ZFAS1* RNA is an antisense transcript against *ZNF1* (Askarian-Amiri and others 2011), a transcription factor, and 3 C/D RNAs (*SNORD12*, *SNORD12B*, and *SNORD12C*) originate from its introns and all guide N_m of a single guanine residue (3,878) in 28S rRNA. *GAS5* locus produces 10 C/D RNAs that guide N_m of different residues in 18S and 28S rRNA. Reduction in *GAS5* expression and *SNORD44* were strongly associated with poor survival in head and neck SCC and breast cancer (Mourtada-Maarabouni and others 2009). Similarly, loss of *SNORD50* expression has been reported in prostate and breast cancers (Dong and others 2008, 2009) while a genetic deletion has been reported in diffuse B cell lymphoma (Tanaka and others 2000). However, *SNORA42* expression is high in non-small-cell lung cancer due to chromosomal amplification and is associated with poor prognosis (Liao and others 2010).

GRIM-1 links IFN action to snoRNA machinery

IFNs were the first cytokines to be developed into therapeutics against certain malignancies and viral diseases. Many solid tumors are refractory to IFN stimulation that can be overcome by pretreatment with retinoids. Retinoids are vitamin A-related compounds that control embryonic and postnatal growth/development in vertebrates. Deficiency of vitamin A leads to night blindness in humans, while animals show a higher cancer incidence. Earlier several preclinical and clinical studies indicated a strong synergistic growth-suppressive effect of retinoid-IFN combination on solid tumors (Kalvakolanu 2004). Using a genome-wide knockdown approach, our laboratory identified a set of genes needed for retinoid-IFN-induced mortality of cancer cells (Hofmann and others 1998; Angell and others 2000) and named them as

genes associated with retinoid-IFN-induced mortality (GRIM). Blockade of expression of any individual GRIMs by an antisense construct confers protection against retinoid-IFN-induced cell death. *GRIM-1* isolated by this approach is involved in regulating snoRNAs and rRNA levels (Hofmann and others 2010) (Fig. 2). *In vitro* translation of *GRIM-1* transcript produces 3 GRIM-1 isoforms (α , β and γ) from the same open reading frame, *ie*, proteins differ in their N-terminal region. Whether a mammalian cell translates *GRIM-1* mRNA in a similar manner is not clear at this stage. However, a polyclonal antibody recognizes bands corresponding to 3 proteins in Western blot analyses. IFN stimulation increases the levels of all isoforms and interestingly, caspase-9 cleaves GRIM-1 α to generate the shorter isoforms (β and γ) that are more apoptotic than GRIM-1 α ; loss of mitochondrial membrane potential appears to activate caspase-9 under such a circumstance (Hofmann and others 2010) (Fig. 2). We have reported earlier that another GRIM, GRIM-19, downregulates the expression of certain antiapoptotic members of the Bcl2 family of proteins. Thus, retinoid-IFN combination can promote mitochondrial permeability transition. Overexpression of individual GRIM-1 isoforms induces growth suppression and apoptosis of cancer cell lines in a differential manner with GRIM1 α and GRIM-1 γ being the weakest and the strongest inducer of apoptosis, respectively. However, a moderate expression is tolerated and cells grow slower than the controls. GRIM-1 isoforms caused a significant drop in *SNORA73* (U17), *SNORA74* (U19), *SNORA50*, *SCARNA8*, *SCARNA13*, *TERC*, snRNA (*RNU2*), and *SNORD3* (U3) levels with a concomitant increase in unprocessed rRNA levels

appear to trigger these events (Nallar and others 2011) (Fig. 2). A converse observation was made upon GRIM-1 depletion from cells using specific shRNAs. Since the shRNA downregulates all GRIM-1 isoforms it does not definitively prove which specific isoform is the most potent inhibitor. Overexpression experiments usually have the caveat of the presence of endogenous isoforms. Hence, we reconstituted the individual RNAi-resistant GRIM-1 constructs into cells that were previously depleted of endogenous GRIM-1. These experiments showed that GRIM-1 γ was the most potent suppressor of sno/scaRNA levels (Nallar and others 2011). In the presence of IFN, DKC1 levels steadily dropped over time and reach a minimum when GRIM-1 protein levels were highest (Kalvakolanu, unpublished). Surprisingly, the NAF1 protein level is unaffected in the same circumstance (Nallar and others 2011). In the presence of GRIM-1 isoforms, NAF1 was primarily found outside the nucleus, indicating that GRIM-1 alters cellular localization of NAF1. The consequence of such effects is lowering snoRNP levels below a threshold. A similar inhibitory effect of GRIM-1 (SHQ1) on H/ACA RNP assembly is evident *in vitro* (Grozdanov and others 2009). Depletion of GRIM-1 levels, by RNAi, conferred a growth advantage in the presence of retinoid-IFN combination and its ectopic expression induces growth suppression of cancer cell lines. Since Shq1p-depleted yeast grew at a slower rate, we tested if yeast Shq1p would act as growth promoter in the mammalian cells. Quite the contrary, upon reconstitution of yeast Shq1p into HeLa cells depleted of the endogenous GRIM-1, expression levels of sno/scaRNAs were suppressed when compared to the controls and significantly suppressed rRNA maturation (Nallar and others 2011). Thus, the functional differences in GRIM-1 action appear to be due to differences in their respective cellular environments, but not their primary sequences. Mammalian cells may have other modifying factors that control GRIM-1 activity, unlike yeast cells. Thus, GRIM-1 appears to be a novel tumor suppressor based on several independent observations: (i) the loss of GRIM-1 expression in prostate cancer specimens by immunohistochemical analysis (Nallar and others 2011), (ii) the monoallelic or biallelic loss in prostate cancers using an integrated oncogenomic approach (Taylor and others 2010), and (iii) the genomic loss in leukemias by high-resolution microarrays (Bullinger and others 2010). A prostate cancer cell line (VCAp) derived from vertebral metastases had very low levels of *GRIM-1* mRNA compared to a noncancerous cell line (RWPE1) (Nallar and others 2011). That an exogenous agent (light signals focused on retina) upregulated GRIM-1 has been independently reported by another study (Schipper and others 2008) wherein the axial ocular growth was halted when chicks experienced an imposed image defocus (using external lenses).

Isolation of several independent genes by our genetic approach suggested a network of noncanonical cell death regulators (GRIM engine) (Kalvakolanu 2004). In this model, a gene product performs a specific task that makes the cancer cell vulnerable to cell death; the strength of each gene product to induce cell death is variable. The loss of mitochondrial membrane potential and/or unregulated reactive oxygen species activate latent caspases to cleave proteins leading to cell death (Fig. 2). Whether caspase-9-mediated GRIM-1 cleavage occurs at steady state is unclear at this stage. Nonetheless, in the presence of IFN, caspase-9

FIG. 2. GRIM-1 is a novel tumor suppressor. Normal cells need a certain level of GRIM-1. IFN can upregulate GRIM-1 levels to cause growth suppression and/or apoptosis by 2 mechanisms: (1) by delocalization of NAF1 and (2) by downregulation of DKC1 levels. IFN-inducible GRIM-1 is cleaved to a potent apoptotic molecule by caspase-9. In the presence of high GRIM-1 levels in the cell, maturation of H/ACA and TERC RNA into RNPs is blocked (thick lines). Some C/D RNAs are also affected in the same circumstance (thin lines). All these are needed to maintain genome stability. GRIM-1 also represses certain snRNPs that are needed for spliceosome function. Blockade of spliceosome can lead to aberrant splicing. GRIM, gene associated with retinoid-IFN-induced mortality; IFN, interferon; RNP, ribonucleoprotein particle.

GRIM-1 into a potent toxic molecule inhibiting snoRNP formation and rRNA maturation (Hofmann and others 2010).

Future perspectives

A recent study showed tissue-specific differences in snoRNA expression levels in normal adult tissues; brain tissues were rich in C/D RNAs and poor in H/ACA RNAs, while in testis, the opposite trend was observed, that is, rich in H/ACA RNAs and poor in C/D RNAs (Castle and others 2010). A few important questions arise in the light of these findings—does this also reflect in a reduction of such modified bases (N_m and Ψ) in a stoichiometric manner in a single rRNA molecule? If yes, how ribosome is fine-tuned when one of the modifications is skewed with respect to the other? Are there distinct pools of ribosomal complexes designated for a specific purpose? Nonetheless, both these snoRNA-guided modifications (N_m and Ψ) have been implicated in resistance to antibiotics as these modifications cluster near functionally important regions on the ribosome/rRNA (Rakauskaite and others 2011). Similarly, gene knockout and mutational studies have shown deficiency and mutants of ribosomal proteins display cell type-specific differences (Anderson and others 2007; Morimoto and others 2007) that prompt a new question—which ribosomal proteins are needed to form the core structural and functional modules of a mammalian ribosome in each tissue. Future studies are required to clarify the function(s) played by an individual or a set of snoRNA(s) in a tissue that normally is not enriched for the same. Similarly, more detailed genetic studies are needed for understanding the impact of the upstream regulators of sno/scaRNAs and their relevance to human cancer. Whether sno/scaRNAs and their regulators also affect the processing of other coding and noncoding RNAs remain to be investigated.

Acknowledgment

D.V.K. is supported by the NIH grant CA105005.

Author Disclosure Statement

No competing financial interests exist.

Abbreviations:

rRNA: Ribosomal RNA representing mature 28S, 18S, and 5.8S species and their precursors
 RNP: Ribonucleoprotein particle
 hnRNA: The precursor for mRNA with some intron(s) still to be excised
 hnRNP: Heterogeneous RNP; plays a role in the cotranscriptional processing of hnRNA
 snRNA: Spliceosomal RNA representing mature U1, U2, U4, U5, and U6 species and their precursors
 snoRNA: Small nucleolar RNA
 snoRNP: A minimal heterotetrameric protein complex containing a snoRNA
 C/D RNA: snoRNA with C (RUGAUGA) and D (uCUGA) box elements that guides 2'-O ribose methylation on rRNA
 C/D RNP: A minimal heterotetrameric protein complex containing a C/D RNA
 H/ACA RNA: snoRNA with a hinge (H-box element; ANANNA) and ACANNN-3'OH motif that guides pseudouridylation on rRNA

H/ACA RNP: A minimal heterotetrameric protein complex containing an H/ACA RNA
 scaRNA: Small Cajal body RNA; a composite group of RNA with a CAB-box element that guides 2'-O ribose methylation or pseudouridylation on snRNA
 scaRNP: A minimal heterotetrameric protein complex containing a scaRNA
 CBs: Cajal bodies; a subnuclear multimolecular mobile platform detectable in cells with moderate to high transcriptional activity. Maturation of snRNA occurs in this structure.
 GRIM: Gene associated with retinoid-IFN-induced mortality.
 SNORD: Gene symbol for a C/D RNA
 SNORA: Gene symbol for an H/ACA RNA
 SCARNA: Gene symbol for a scaRNA
 TERC: Telomerase RNA component; a special sno/scaRNA needed for Telomerase function
 TERT: Telomerase reverse transcriptase; the enzymatic component of Telomerase holoenzyme
 m^N : Methylated nucleobase ($N = A$ or C in RNA; dA or dC in DNA)
 N_m : 2'-O ribose methylation or methylated nucleotide ($N = A, G, C$ or U)
 Ψ : Pseudouridine or pseudouridylation; changing a N-glycoside to a C-glycoside in RNA
 miRNA: Micro RNA

References

- Anderson SJ, Lauritsen JP, Hartman MG, Foushee AM, Lefebvre JM, et al. 2007. Ablation of ribosomal protein L22 selectively impairs alphabeta T cell development by activation of a p53-dependent checkpoint. *Immunity* 26:759–772.
- Angell JE, Lindner DJ, Shapiro PS, Hofmann ER, Kalvakolanu DV. 2000. Identification of GRIM-19, a novel cell death-regulatory gene induced by the interferon-beta and retinoic acid combination, using a genetic approach. *J Biol Chem* 275:33416–33426.
- Angrisani A, Turano M, Paparo L, Di Mauro C, Furia M. 2011. A new human dyskerin isoform with cytoplasmic localization. *Biochim Biophys Acta* 1810:1361–1368.
- Appaiah HN, Goswami CP, Mina LA, Badve S, Sledge GW, Jr., et al. 2011. Persistent upregulation of U6:SNORD44 small RNA ratio in the serum of breast cancer patients. *Breast Cancer Res* 13:R86.
- Askarian-Amiri ME, Crawford J, French JD, Smart CE, Smith MA, et al. 2011. SNORD-host RNA Zfas1 is a regulator of mammary development and a potential marker for breast cancer. *RNA* 17:878–891.
- Balakin AG, Smith L, Fournier MJ. 1996. The RNA world of the nucleolus: two major families of small RNAs defined by different box elements with related functions. *Cell* 86:823–834.
- Belin S, Beghin A, Solano-Gonzalez E, Bezin L, Brunet-Manquat S, et al. 2009. Dysregulation of ribosome biogenesis and translational capacity is associated with tumor progression of human breast cancer cells. *PLoS One* 4:e7147.
- Bellodi C, Kopmar N, Ruggero D. 2010a. Dereglulation of oncogene-induced senescence and p53 translational control in X-linked dyskeratosis congenita. *EMBO J* 29:1865–1876.
- Bellodi C, Krasnykh O, Haynes N, Theodoropoulou M, Peng G, et al. 2010b. Loss of function of the tumor suppressor DKC1 perturbs p27 translation control and contributes to pituitary tumorigenesis. *Cancer Res* 70:6026–6035.

- Bodi Z, Zhong S, Mehra S, Song J, Graham N, et al. 2012. Adenosine methylation in Arabidopsis mRNA is associated with the 3' end and reduced levels cause developmental defects. *Front Plant Sci* 3:48.
- Bojovic B, Crowe DL. 2011. Telomere dysfunction promotes metastasis in a TERC null mouse model of head and neck cancer. *Mol Cancer Res* 9:901–913.
- Boulon S, Verheggen C, Jady BE, Girard C, Pescia C, et al. 2004. PHAX and CRM1 are required sequentially to transport U3 snoRNA to nucleoli. *Mol Cell* 16:777–787.
- Bryan TM, Englezou A, Dalla-Pozza L, Dunham MA, Reddel RR. 1997. Evidence for an alternative mechanism for maintaining telomere length in human tumors and tumor-derived cell lines. *Nat Med* 3:1271–1274.
- Bullinger L, Kronke J, Schon C, Radtke I, Urlbauer K, et al. 2010. Identification of acquired copy number alterations and uniparental disomies in cytogenetically normal acute myeloid leukemia using high-resolution single-nucleotide polymorphism analysis. *Leukemia* 24:438–449.
- Castle JC, Armour CD, Lower M, Haynor D, Biery M, et al. 2010. Digital genome-wide ncRNA expression, including SnoRNAs, across 11 human tissues using polyA-neutral amplification. *PLoS One* 5:e11779.
- Chaneton B, Gottlieb E. 2012. Rocking cell metabolism: revised functions of the key glycolytic regulator PKM2 in cancer. *Trends Biochem Sci* 37:309–316.
- Chen HH, Chang JG, Lu RM, Peng TY, Tarn WY. 2008. The RNA binding protein hnRNP Q modulates the utilization of exon 7 in the survival motor neuron 2 (SMN2) gene. *Mol Cell Biol* 28:6929–6938.
- Clower CV, Chatterjee D, Wang Z, Cantley LC, Vander Heiden MG, et al. 2010. The alternative splicing repressors hnRNP A1/A2 and PTB influence pyruvate kinase isoform expression and cell metabolism. *Proc Natl Acad Sci U S A* 107:1894–1899.
- Collins K, Mitchell JR. 2002. Telomerase in the human organism. *Oncogene* 21:564–579.
- Creagan RP, Tan YH, Chen S, Ruddle FH. 1975. Somatic cell genetic analysis of the interferon system. *Fed Proc* 34:2222–2226.
- Decatur WA, Fournier MJ. 2002. rRNA modifications and ribosome function. *Trends Biochem Sci* 27:344–351.
- Decatur WA, Fournier MJ. 2003. RNA-guided nucleotide modification of ribosomal and other RNAs. *J Biol Chem* 278:695–698.
- de los Santos T, Schweizer J, Rees CA, Francke U. 2000. Small evolutionarily conserved RNA, resembling C/D box small nucleolar RNA, is transcribed from PWCR1, a novel imprinted gene in the Prader-Willi deletion region, which is highly expressed in brain. *Am J Hum Genet* 67:1067–1082.
- Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, et al. 2012. Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. *Nature* 485:201–206.
- Dong XY, Guo P, Boyd J, Sun X, Li Q, et al. 2009. Implication of snoRNA U50 in human breast cancer. *J Genet Genomics* 36:447–454.
- Dong XY, Rodriguez C, Guo P, Sun X, Talbot JT, et al. 2008. SnoRNA U50 is a candidate tumor-suppressor gene at 6q14.3 with a mutation associated with clinically significant prostate cancer. *Hum Mol Genet* 17:1031–1042.
- Donsante A, Miller DG, Li Y, Vogler C, Brunt EM, et al. 2007. AAV vector integration sites in mouse hepatocellular carcinoma. *Science* 317:477.
- Dunn GP, Koebel CM, Schreiber RD. 2006. Interferons, immunity and cancer immunoediting. *Nat Rev Immunol* 6:836–848.
- Eliceiri GL. 2006. The vertebrate E1/U17 small nucleolar ribonucleoprotein particle. *J Cell Biochem* 98:486–495.
- Ender C, Krek A, Friedlander MR, Beitzinger M, Weinmann L, et al. 2008. A human snoRNA with microRNA-like functions. *Mol Cell* 32:519–528.
- Enninga J, Levy DE, Blobel G, Fontoura BM. 2002. Role of nucleoporin induction in releasing an mRNA nuclear export block. *Science* 295:1523–1525.
- Flomen R, Knight J, Sham P, Kerwin R, Makoff A. 2004. Evidence that RNA editing modulates splice site selection in the 5-HT2C receptor gene. *Nucleic Acids Res* 32:2113–2122.
- Fromont-Racine M, Senger B, Saveanu C, Fasiolo F. 2003. Ribosome assembly in eukaryotes. *Gene* 313:17–42.
- Ge J, Rudnick DA, He J, Crimmins DL, Ladenson JH, et al. 2010. Dyskerin ablation in mouse liver inhibits rRNA processing and cell division. *Mol Cell Biol* 30:413–422.
- Gee HE, Buffa FM, Camps C, Ramachandran A, Leek R, et al. 2011. The small-nucleolar RNAs commonly used for microRNA normalisation correlate with tumour pathology and prognosis. *Br J Cancer* 104:1168–1177.
- Gewin L, Myers H, Kiyono T, Galloway DA. 2004. Identification of a novel telomerase repressor that interacts with the human papillomavirus type-16 E6/E6-AP complex. *Genes Dev* 18:2269–2282.
- Gordon FE, Nutt CL, Cheunsuchon P, Nakayama Y, Provencher KA, et al. 2010. Increased expression of angiogenic genes in the brains of mouse meg3-null embryos. *Endocrinology* 151:2443–2452.
- Grozdanov PN, Roy S, Kittur N, Meier UT. 2009. SHQ1 is required prior to NAF1 for assembly of H/ACA small nucleolar and telomerase RNPs. *RNA* 15:1188–1197.
- Gu T, He H, Han Z, Zeng T, Huang Z, et al. 2012. Expression of macro non-coding RNAs Meg8 and Irm in mouse embryonic development. *Acta Histochem* 114:392–399.
- Guo J, Hui DJ, Merrick WC, Sen GC. 2000. A new pathway of translational regulation mediated by eukaryotic initiation factor 3. *EMBO J* 19:6891–6899.
- He J, Navarrete S, Jasinski M, Vulliamy T, Dokal I, et al. 2002. Targeted disruption of Dkc1, the gene mutated in X-linked dyskeratosis congenita, causes embryonic lethality in mice. *Oncogene* 21:7740–7744.
- Hofmann ER, Boyanapalli M, Lindner DJ, Weihua X, Hassel BA, et al. 1998. Thioredoxin reductase mediates cell death effects of the combination of beta interferon and retinoic acid. *Mol Cell Biol* 18:6493–6504.
- Hofmann ER, Nallar SC, Lin L, D'Cunha J, Lindner DJ, et al. 2010. Identification and characterization of GRIM-1, a cell-death-associated gene product. *J Cell Sci* 123:2781–2791.
- Isaac C, Yang Y, Meier UT. 1998. Nopp140 functions as a molecular link between the nucleolus and the coiled bodies. *J Cell Biol* 142:319–329.
- Jiang YH, Armstrong D, Albrecht U, Atkins CM, Noebels JL, et al. 1998. Mutation of the Angelman ubiquitin ligase in mice causes increased cytoplasmic p53 and deficits of contextual learning and long-term potentiation. *Neuron* 21:799–811.
- Kalvakolanu DV. 2004. The GRIMs: a new interface between cell death regulation and interferon/retinoid induced growth suppression. *Cytokine Growth Factor Rev* 15:169–194.
- Kato L, Begum NA, Burroughs AM, Doi T, Kawai J, et al. 2012. Nonimmunoglobulin target loci of activation-induced cytidine deaminase (AID) share unique features with immunoglobulin genes. *Proc Natl Acad Sci U S A* 109:2479–2484.

- King TH, Decatur WA, Bertrand E, Maxwell ES, Fournier MJ. 2001. A well-connected and conserved nucleoplasmic helicase is required for production of box C/D and H/ACA snoRNAs and localization of snoRNP proteins. *Mol Cell Biol* 21:7731–7746.
- Kirwan M, Dokal I. 2008. Dyskeratosis congenita: a genetic disorder of many faces. *Clin Genet* 73:103–112.
- Kishore S, Khanna A, Zhang Z, Hui J, Balwierz PJ, et al. 2010. The snoRNA MBII-52 (SNORD 115) is processed into smaller RNAs and regulates alternative splicing. *Hum Mol Genet* 19:1153–1164.
- Kishore S, Stamm S. 2006. The snoRNA HBII-52 regulates alternative splicing of the serotonin receptor 2C. *Science* 311:230–232.
- Kiss T, Fayet E, Jady BE, Richard P, Weber M. 2006. Biogenesis and intranuclear trafficking of human box C/D and H/ACA RNPs. *Cold Spring Harb Symp Quant Biol* 71:407–417.
- Li H, Tai BC. 2006. RNASEL gene polymorphisms and the risk of prostate cancer: a meta-analysis. *Clin Cancer Res* 12: 5713–5719.
- Liao J, Yu L, Mei Y, Guarnera M, Shen J, et al. 2010. Small nucleolar RNA signatures as biomarkers for non-small-cell lung cancer. *Mol Cancer* 9:198.
- Marinus MG, Morris NR. 1973. Isolation of deoxyribonucleic acid methylase mutants of *Escherichia coli* K-12. *J Bacteriol* 114:1143–1150.
- Mazurek S, Boschek CB, Hugo F, Eigenbrodt E. 2005. Pyruvate kinase type M2 and its role in tumor growth and spreading. *Semin Cancer Biol* 15:300–308.
- Mei YP, Liao JP, Shen J, Yu L, Liu BL, et al. 2011. Small nucleolar RNA 42 acts as an oncogene in lung tumorigenesis. *Oncogene* 31:2794–2804.
- Meyer KD, Saletore Y, Zumbo P, Elemento O, Mason CE, et al. 2012. Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. *Cell* 149:1635–1646.
- Michel CI, Holley CL, Scruggs BS, Sidhu R, Brookheart RT, et al. 2011. Small nucleolar RNAs U32a, U33, and U35a are critical mediators of metabolic stress. *Cell Metab* 14:33–44.
- Mishra RK, Eliceiri GL. 1997. Three small nucleolar RNAs that are involved in ribosomal RNA precursor processing. *Proc Natl Acad Sci U S A* 94:4972–4977.
- Montanaro L, Calienni M, Bertoni S, Rocchi L, Sansone P, et al. 2010. Novel dyskerin-mediated mechanism of p53 inactivation through defective mRNA translation. *Cancer Res* 70: 4767–4777.
- Morimoto K, Lin S, Sakamoto K. 2007. The functions of RPS19 and their relationship to Diamond-Blackfan anemia: a review. *Mol Genet Metab* 90:358–362.
- Mourtada-Maarabouni M, Pickard MR, Hedge VL, Farzaneh F, Williams GT. 2009. GAS5, a non-protein-coding RNA, controls apoptosis and is downregulated in breast cancer. *Oncogene* 28:195–208.
- Mukhopadhyay R, Ray PS, Arif A, Brady AK, Kinter M, et al. 2008. DAPK-ZIPK-L13a axis constitutes a negative-feedback module regulating inflammatory gene expression. *Mol Cell* 32:371–382.
- Nallar SC, Lin L, Srivastava V, Gade P, Hofmann ER, et al. 2011. GRIM-1, a novel growth suppressor, inhibits rRNA maturation by suppressing small nucleolar RNAs. *PLoS One* 6:e24082.
- Nicholls RD, Knepper JL. 2001. Genome organization, function, and imprinting in Prader-Willi and Angelman syndromes. *Annu Rev Genomics Hum Genet* 2:153–175.
- Noguchi T, Inoue H, Tanaka T. 1986. The M1- and M2-type isozymes of rat pyruvate kinase are produced from the same gene by alternative RNA splicing. *J Biol Chem* 261:13807–13812.
- Ofengand J, Bakin A. 1997. Mapping to nucleotide resolution of pseudouridine residues in large subunit ribosomal RNAs from representative eukaryotes, prokaryotes, archaeobacteria, mitochondria and chloroplasts. *J Mol Biol* 266:246–268.
- Pelczar P, Filipowicz W. 1998. The host gene for intronic U17 small nucleolar RNAs in mammals has no protein-coding potential and is a member of the 5'-terminal oligopyrimidine gene family. *Mol Cell Biol* 18:4509–4518.
- Pellizzoni L, Baccon J, Charroux B, Dreyfuss G. 2001. The survival of motor neurons (SMN) protein interacts with the snoRNP proteins fibrillarin and GAR1. *Curr Biol* 11:1079–1088.
- Rakauskaite R, Dinman JD. 2011. Mutations of highly conserved bases in the peptidyltransferase center induce compensatory rearrangements in yeast ribosomes. *RNA* 17:855–864.
- Richard P, Darzacq X, Bertrand E, Jady BE, Verheggen C, et al. 2003. A common sequence motif determines the Cajal body-specific localization of box H/ACA scaRNAs. *EMBO J* 22:4283–4293.
- Roy NS, Nakano T, Keyoung HM, Windrem M, Rashbaum WK, et al. 2004. Telomerase immortalization of neuronally restricted progenitor cells derived from the human fetal spinal cord. *Nat Biotechnol* 22:297–305.
- Ruddle FH, Creagan RP. 1975. Parasexual approaches to the genetics of man. *Annu Rev Genet* 9:407–486.
- Rudolph KL, Chang S, Lee HW, Blasco M, Gottlieb GJ, et al. 1999. Longevity, stress response, and cancer in aging telomerase-deficient mice. *Cell* 96:701–712.
- Ruggero D, Grisendi S, Piazza F, Rego E, Mari F, et al. 2003. Dyskeratosis congenita and cancer in mice deficient in ribosomal RNA modification. *Science* 299:259–262.
- Runte M, Varon R, Horn D, Horsthemke B, Buiting K. 2005. Exclusion of the C/D box snoRNA gene cluster HBII-52 from a major role in Prader-Willi syndrome. *Hum Genet* 116:228–230.
- Sahoo T, del Gaudio D, German JR, Shinawi M, Peters SU, et al. 2008. Prader-Willi phenotype caused by paternal deficiency for the HBII-85 C/D box small nucleolar RNA cluster. *Nat Genet* 40:719–721.
- Samuel CE. 1979. Mechanism of interferon action: phosphorylation of protein synthesis initiation factor eIF-2 in interferon-treated human cells by a ribosome-associated kinase processing site specificity similar to hemin-regulated rabbit reticulocyte kinase. *Proc Natl Acad Sci U S A* 76:600–604.
- Scheffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM. 1990. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. *Cell* 63:1129–1136.
- Schippert R, Schaeffel F, Feldkaemper MP. 2008. Microarray analysis of retinal gene expression in chicks during imposed myopic defocus. *Mol Vis* 14:1589–1599.
- Scott MS, Avolio F, Ono M, Lamond AI, Barton GJ. 2009. Human miRNA precursors with box H/ACA snoRNA features. *PLoS Comput Biol* 5:e1000507.
- Silvera D, Formenti SC, Schneider RJ. 2010. Translational control in cancer. *Nat Rev Cancer* 10:254–266.
- Song S, Pitot HC, Lambert PF. 1999. The human papillomavirus type 16 E6 gene alone is sufficient to induce carcinomas in transgenic animals. *J Virol* 73:5887–5893.
- Stadtfield M, Apostolou E, Akutsu H, Fukuda A, Follett P, et al. 2010. Aberrant silencing of imprinted genes on chromosome 12qF1 in mouse induced pluripotent stem cells. *Nature* 465: 175–181.
- Stefan M, Simmons RA, Bertera S, Trucco M, Esni F, et al. 2011. Global deficits in development, function, and gene expression

- in the endocrine pancreas in a deletion mouse model of Prader-Willi syndrome. *Am J Physiol Endocrinol Metab* 300: E909–E922.
- Tanaka R, Satoh H, Moriyama M, Satoh K, Morishita Y, et al. 2000. Intronic U50 small-nucleolar-RNA (snoRNA) host gene of no protein-coding potential is mapped at the chromosome breakpoint t(3;6)(q27;q15) of human B-cell lymphoma. *Genes Cells* 5:277–287.
- Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, et al. 2010. Integrative genomic profiling of human prostate cancer. *Cancer Cell* 18:11–22.
- Terns M, Terns R. 2006. Noncoding RNAs of the H/ACA family. *Cold Spring Harb Symp Quant Biol* 71:395–405.
- Tu W, Xu X, Peng L, Zhong X, Zhang W, et al. 2010. DAPK1 interaction with NMDA receptor NR2B subunits mediates brain damage in stroke. *Cell* 140:222–234.
- Tyc K, Steitz JA. 1989. U3, U8 and U13 comprise a new class of mammalian snRNPs localized in the cell nucleolus. *EMBO J* 8:3113–3119.
- Vulliamy TJ, Dokal I. 2008. Dyskeratosis congenita: the diverse clinical presentation of mutations in the telomerase complex. *Biochimie* 90:122–130.
- Wilson GG. 1991. Organization of restriction-modification systems. *Nucleic Acids Res* 19:2539–2566.
- Wu G, Xiao M, Yang C, Yu YT. 2011. U2 snRNA is inducibly pseudouridylated at novel sites by Pus7p and snR81 RNP. *EMBO J* 30:79–89.
- Wu H, Feigon J. 2007. H/ACA small nucleolar RNA pseudouridylation pockets bind substrate RNA to form three-way junctions that position the target U for modification. *Proc Natl Acad Sci U S A* 104:6655–6660.
- Yang PK, Rotondo G, Porras T, Legrain P, Chanfreau G. 2002. The Shq1p.Naf1p complex is required for box H/ACA small nucleolar ribonucleoprotein particle biogenesis. *J Biol Chem* 277:45235–45242.
- Zhang J, Harnpicharnchai P, Jakovljevic J, Tang L, Guo Y, et al. 2007. Assembly factors Rpf2 and Rrs1 recruit 5S rRNA and ribosomal proteins rpL5 and rpL11 into nascent ribosomes. *Genes Dev* 21:2580–2592.
- Zhou A, Hassel BA, Silverman RH. 1993. Expression cloning of 2–5A-dependent RNAase: a uniquely regulated mediator of interferon action. *Cell* 72:753–765.
- Zhou Y, Zhang X, Klibanski A. 2012. MEG3 noncoding RNA: a tumor suppressor. *J Mol Endocrinol* 48:R45–R53.
- Zhou Y, Zhong Y, Wang Y, Zhang X, Batista DL, et al. 2007. Activation of p53 by MEG3 non-coding RNA. *J Biol Chem* 282:24731–24742.
- Zhu PJ, Huang W, Kalikulov D, Yoo JW, Placzek AN, et al. 2011. Suppression of PKR promotes network excitability and enhanced cognition by interferon-gamma-mediated disinhibition. *Cell* 147:1384–1396.

Address correspondence to:
 Prof. Dhananjaya V. Kalvakolanu
 Department of Microbiology & Immunology
 Greenebaum Cancer Center
 University of Maryland School of Medicine
 Baltimore, MD 21201

E-mail: dkalvako@umaryland.edu

Received 6 September 2012/Accepted 6 October 2012