Chapter 9

Long Noncoding Mitochondrial RNAs (LncmtRNAs) as Targets for Cancer Therapy

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Abstract

Mitochondria are traditionally been viewed as the cell's powerhouse, generating most of its ATP. However, besides this fundamental metabolic role, mitochondria are implicated in diverse other processes, including apoptosis, inflammation and metastasis. These functions are exerted in part by the growing class of long noncoding mitochondrial RNAs (lncmtRNAs). We found that normal human proliferating cells express a family of noncoding mitochondrial RNAs (ncmtRNAs), comprised of sense (SncmtRNA) and antisense (ASncmtRNA). However, tumor cells express only sense transcripts, suggesting that ASncmtRNA downregulation as a cancer new hallmark. The few ASncmtRNAs copies in tumor cells seem essential to tumor cell viability: knockdown of these transcripts with antisense oligonucleotides (ASO) causes massive apoptotic death of tumor cells, preceded by cell cycle arrest. Preclinical assays show that systemic administration of ASO delayed tumor growth in melanoma and renal cancer models and, caused total remission in subcutaneous renal cancer tumors. The same treatment, however, does not affect normal tissue, suggesting this approach for the development of an efficient and safe therapeutic strategy for several cancer types.

Keywords: cancer, mitochondria, long noncoding RNAs, antisense oligonucleotides, therapy

1. Introduction

Mitochondria are eukaryotic cell organelles that represent a universal system in higher organisms which generate most of the cellular energy, in the form of ATP, necessary for different

cell processes. However, many other functions have been assigned to this tiny organelle, such as apoptosis, reactive oxidative species (ROS) signaling, inflammation and metastasis. Mitochondria play a central role in apoptosis, principally due to the release of proteins from the mitochondrial inter-membrane space [1], linking mitochondria to cell suicide. Mitochondria also represent a major source of DNA-damaging reactive oxygen species (ROS), mainly as by-products of oxidative phosphorylation. In comparison to nuclear DNA, mitochondrial DNA (mtDNA) is more susceptible to DNA damage due to the reduced capacity of the cell to repair mtDNA, potentially promoting cancer [2].

In inflammation, mitochondria mainly serve as a source of various signaling molecules, termed damage-associated molecular patterns or DAMPS, which propagate inflammatory signals and therefore activate inflammation [3].

Cancer death is most often due to secondary tumors or metastasis. This process requires a complex series of events, which include epithelial-to-mesenchymal transition, stromal remodeling, invasion and ultimately migration of cancer cells. In this context, mitochondrial ROS play a key role, leading to angiogenesis and metastasis [4] and promoting the migratory plasticity of cells through activation of two essential factors, Src and protein tyrosine kinase 2 [5]. The basic understanding of the dependence of cancer cells on various mitochondrial roles is already endorsing novel therapeutic approaches in cancer.

Along that line, an expanding group of evidences indicate that the mitochondrial genome is not only responsible for the synthesis of the canonical 13 proteins, 22 tRNAs and two ribosomal RNAs (12S and 16S). Novel and expanding evidence suggest that mtDNA compensates for reduced length by using little known phenomena that potentially increase DNA's protein coding repertoire, such as so called swinger polymerization, that consists of systematic exchanges between nucleotides during DNA or RNA polymerization, producing so-called swinger sequences [6, 7]. These transformations alter gene and mRNA coding properties. Moreover, in human mitochondria systematic deletions of mono- and dinucleotides after each trinucleotide have been reported, producing delRNAs. Recently, an exhaustive analysis of human nanoLc mass spectrometry peptidome data detect numerous tetra- and pentapeptides translated from the human mitogenome, and this peptide subgroup would be the result of the translation of delRNAs. Therefore, non-canonical transcriptions and translations could considerably expand the coding potential of mitochondrial DNA and RNA sequences [8, 9].

Finally, in the field of antisense RNAs and because mitochondrial tRNA mutations are 6.5 times more frequently pathogenic than in other mitochondrial sequences, a potential additional tRNA gene function is that of templating for antisense tRNAs. Most antisense tRNAs probably function routinely in translation and extend the tRNA pool and mutation pathogenicity, probably frequently resulting from a mixture of effects due to sense and antisense tRNA translational activity for many mitochondrial tRNAs [10, 11]. These could link to mitochondrial disorders and cancer.

This small but powerful organelle is also a novel source of noncoding RNAs (ncRNAs), and growing evidence shows that mammalian mitochondria can also import/export ncRNAs, turning this organelle into a pivotal player not only in cellular physiology but also in cancer, representing potential targets for innovative ncRNA-based treatment strategies [12].

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In this chapter we discuss the importance of long noncoding mitochondrial RNAs (lncmtRNAs) in diagnostic and pharmaceutical targeting in cancer.

2. Mitochondrial transcripts and noncoding RNAs

Human mitochondrial DNA (mtDNA) is a circular molecule of 16,569 bp in length [13] which encodes a small subset of 13 proteins required for OxPhos, 22 tRNAs and two ribosomal RNAs, 12S rRNA and 16S rRNA, which form part of the small (28S) and large (39S) subunits of the 55S mitoribosome [14]. All other protein components are encoded by nuclear genes and imported into mitochondria from the cytosol.

Replication and transcription of mtDNA is initiated from the D loop, a small noncoding region, and is regulated by nuclear-encoded proteins imported into mitochondria [15]. Mitochondrial RNAs are transcribed as long polycistronic precursors from both strands, termed heavy (H) and light (L) strands [16]. Except for NADH dehydrogenase 6 (ND6), all the 13-mitochondrial proteins are encoded in the H-strand. Additionally, the H-strand encodes 14 of the 22 tRNAs and the 2 rRNAs. The remaining 8 tRNAs are encoded on the L-strand [17]. The precursor transcripts are processed according to the tRNA punctuation model, whereby 22 interspersed tRNAs are excised at their 5′ and 3′ends by RNase P [18] and by RNase Z, elaC homology 2 (ELAC2), respectively, releasing simultaneously individual rRNAs and mRNAs [19]. The RNAs then undergo maturation, involving polyadenilation at the 3′eextremitoess of mRNAs and rRNAs, and specific nucleotide modifications and addition of CCA trinucleotides to the 3′eextremities of tRNAs [20]. The data of several groups indicate that 250–300 nuclear-encoded proteins are dedicated exclusively to serve mitochondrial gene expression. This includes RNA polymerase, endonucleases for RNA processing, translation factors, biogenesis factors for the mitochondrial ribosome, aminoacyl-tRNA synthetases, and other auxiliary factors [21, 22].

However, evidence has accumulated supporting the notion that, besides proteins, many types of RNAs transcribed from the nuclear genome are actively delivered to mitochondria. Among these transcripts are different types of noncoding RNAs, such as tRNAs, 5S rRNA, MRP RNA (RMRP) and RNase P RNA (RPPH1) [23], as well as microRNAs (mitomiRs) [24]. The logical explanation is that, despite their critical function, the handfuls of mitochondrial- and nuclear-encoded proteins are insufficient to maintain mitochondrial structure or activity.

Noncoding RNAs (ncRNAs) are divided in two major groups according to size, as small noncoding RNAs and long noncoding RNAs. Among the small ncRNAs, microRNAs (miRNAs) are the most-studied class in mammals. These RNAs (20–24 nucleotides in length) negatively regulate gene expression through binding with their target mRNA and have been implicated actively in pathogenic processes of many human diseases [24] and, as such, are important regulators of cancer cell metabolism [25]. The observation of association of miRNAs with/inside mitochondria may have important implications in several cellular processes and suggest that the role of mitochondria clearly extends beyond its role in energy metabolism and other cellular processes. The newfound destination of miRNAs indicates novel roles of mitochondria in normal and pathological events [25].

The class of long noncoding RNAs (lncRNAs) has been recently recognized, and is defined as transcripts longer than 200 nucleotides. The size cutoff is arbitrary and many functional lncRNAs are considerably longer than 200 nucleotides, including X-inactive specific transcript (XIST) [26], its antisense form Tsix [27], and Hox transcript antisense intergenic RNA (HOTAIR) [28], which are several kilobases (kb) in length.

LncRNAs are able to interact with DNA, RNA, and proteins. In doing so, they regulate several processes including chromatin dynamics, gene transcription, splicing, and translation [29]. Their involvement in these processes implicates lncRNAs in various aspects of human physiology and disease, which include cancer. Aberrant lncRNA expression has been associated to various cancer types [30]. Moreover, deregulated lncRNA expression patterns can modulate several hallmarks of cancer [31], including sustained growth signaling, repressed growth inhibition, apoptosis evasion, stimulated proliferation, and the promotion of angiogenesis [32].

2.1. Long noncoding RNAs are generated in the mitochondria

Both strands of the mtDNA are entirely transcribed but the light strand carries only genes for seven tRNAs and the ND6 protein. Therefore, large noncoding sequences are thus generated and released upon transcript processing. Three lncRNAs generated from mtDNA transcription have been proposed. Their presence was authenticated by Northern blot and qRT-PCR analysis. These transcripts are complementary to MTND5, MTND6 and MTCYTB genes. These molecules form intermolecular duplexes that resist RNase 1 digestion, suggesting regulation of their complementary coding mRNA. Therefore, pairing of these lncRNAs with their mRNA targets might, for instance, control translation [33].

Recently, a novel mitochondrial long noncoding RNA (mtlncRNA) was identified in the plasma of patients with left ventricular (LV) remodeling post-myocardial infarction. Levels of LIPCAR (long intergenic noncoding RNA predicting cardiac remodeling) decline in early stages after myocardial infarction, but increase in late stages, coinciding with LV remodeling. Therefore, high levels of LIPCAR associate with identified patients with high risk of heart failure, even death, suggesting that this lncmtRNA as a potential biomarker for patients with recent episodes of acute myocardial infarction [34].

In the year 2000, our laboratory described a novel chimeric mitochondrial RNA present in mouse testis and sperm cells. This transcript contained an inverted repeat of 120nt joined to the 5′ end of the 16S mitochondrial rRNA. The presence of this novel mitochondrial RNA in sperm, testis, and somatic tissues was demonstrated by RT-PCR. As to the origin of this novel RNA, one possibility was that it arose from transcription altered mtDNA which contained an insert of 121bp between the tRNAVal and the 16S rRNA genes. However, PCR of sperm, testis, liver, and blood cell mtDNA between these two genes yielded a fragment of 342bp consistent with a normal mtDNA lacking the putative insertion of 121bp. The most surprising result was the localization of this novel RNA in the sperm nucleus. In situ hybridization (ISH) demonstrated that the sequence of the 16S rRNA and that of the inverted repeat were localized in the sperm nucleus [35].

Nuclear localization of this mitochondrial transcript is not specific to mouse since, by ISH, we found that at least the sequence of the 16S rRNA was also localized in the nucleus of human

sperm. To determine when during spermatogenesis the mitochondrial RNA is localized in the nucleus, ISH of mouse and human testis was carried out. The nuclei of spermatogonia, spermatocytes and round and elongated spermatids were all positively stained. In human spermatocytes, the nuclear staining pattern was fibrillar, suggesting an association of the mitochondrial transcript with the meiotic chromosomes [36].

These results suggested that the nuclear localization of the 16S mitochondrial rRNA in spermatogenic cells is the result of an intriguing process of translocation of the transcript from the organelle to the nucleus. This hypothesis leads to several important questions; for example, how does the organelle regulate the exit of the 16S mitochondrial rRNA without affecting the number of copies needed to assemble mitoribosomes for normal mitochondrial translation? Or what is the mechanism by which this RNA is exported from mitochondria? At present these questions remain unanswered.

However, the extramitochondrial localization of the 16S mitochondrial rRNA described by us is not unique, since the same transcript has been found consistently in the cytoplasm of Drosophila and Xenopus embryos [37, 38]. Moreover, injection of an anti-16S rRNA ribozyme into cleavage embryos of Drosophila demonstrated that the rRNA is actively involved in the generation of pole cells, progenitors of the germ line [39].

As mentioned above, ISH revealed that this lncRNA is over-expressed in human sperm and precursor cells [36]. These results suggest that human cells might contain a transcript with structural features similar to the mouse RNA. We found that the human RNA is over-expressed in several human proliferating cells but not in resting cells. The structure of this transcript of 2374 nt, which we designated sense noncoding mitochondrial RNA or SncmtRNA, revealed the presence of an inverted repeat (IR) of 815 nt linked to the 5′ end of the 16S mtrRNA. The expression of this transcript can be induced in resting lymphocytes stimulated with phytohaemagglutinin (PHA), together with DNA synthesis and the expression of the proliferation markers proliferating cell nuclear antigen (PCNA), Ki-67 and phosphohistone H3. On the other hand, treatment of DU145 cells with aphidicolin reversibly blocks cell proliferation as well as the expression of the ncmtRNA. These results suggested that the ncmtRNA is a new marker of cell proliferation [40].

Afterwards, we described 2 mitochondrial transcripts (ASncmtRNAs) in human cells containing stem-loop structures similar to that of the previously described SncmtRNA. Regarding expression of the SncmtRNA and the ASncmtRNAs, 3 different phenotypes of human cells can be defined. Normal proliferating cells express both families of transcripts; in striking contrast, tumor cells express the SncmtRNA and down-regulate the ASncmtRNAs. Finally, neither of these transcripts is expressed in nondividing cells. Down-regulation of the ASncmtRNAs was observed in 15 different tumor cell lines and in tumor cells present in 273 cancer biopsies corresponding to 17 different cancer types [41]. SncmtRNA is expressed in all proliferating cells, independently whether we are dealing with a regulated or a dysfunctional cell cycle. The fact that the ASncmtRNAs are always down-regulated in tumor cells suggests that, hypothetically, the ASncmtRNAs might function as a unique mitochondria-encoded tumor suppressor. **Figure 1** shows a panel of ISH for S and ASncmtRNA in non-proliferating tissue (liver), tumor tissue (cervix carcinoma) and normal proliferating tissue (normal cervix epithelium), representing the concept of differential expression mentioned. For in situ hybridization, tissue sections were incubated with hybridization mixture

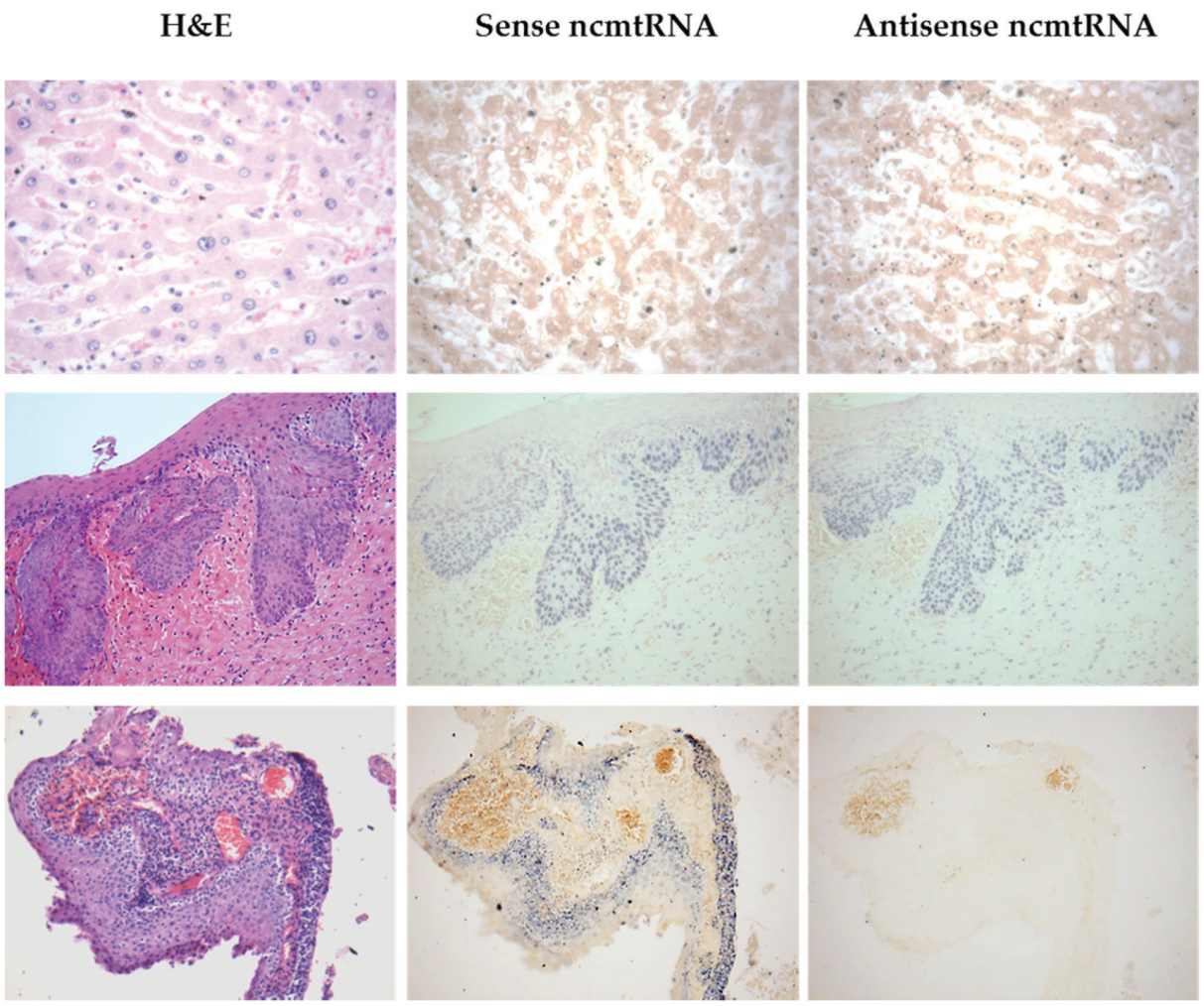

Figure 1. Representative in situ hybridization assay showing the differential expression pattern of lncmtRNAs in tissues according to proliferative status. Upper panel shows absence of signal for both RNAs in non-proliferating tissues such as liver. Middle panel shows presence of strong punctuate signal, corresponding to nuclei in normal proliferating cervix epithelium. Lower panel shows a strong signal corresponding only to SncmtRNA and complete absence of signal corresponding to ASncmtRNA in a tumor tissue, exemplified by cervix carcinoma. H&E, hematoxylin-eosin staining. Magnification in upper panel, ×200. Magnification in cervix tissues, ×100.

containing probes complementary to sense or antisense ncmtRNAs, previously labeled at the 3′ end with digoxigenin-11-dUTP (Boehringer Mannheim, Germany) as described previously [36]. For detection, sections were incubated with a monoclonal anti-digoxigenin antibody conjugated to alkaline phosphatase, and after color development, positive signal correspond to a blue color, representing the expression of the corresponding RNA (see **Figure 1**).

Regarding subcellular localization, we found that in biopsies of normal and cancer tissues, nuclear localization of these transcripts was frequently observed. The extra-mitochondrial localization of these transcripts was confirmed by electron microscopy ISH. In normal cells, SncmtRNA and the ASncmtRNAs were found in the nucleus associated to chromatin. In tumor cells, SncmtRNA shows similar localization plus association with nucleoli, while the ASncmtRNAs are down-regulated. Although the meaning of the nuclear localization in normal proliferating cells of SncmtRNA and the ASncmtRNAs is unclear, the results suggest that these

transcripts might play a role in retrograde signaling. Down regulation of the ASncmtRNAs seems to be an important step in neoplastic transformation and cancer progression [42].

The study of RNA and DNA oncogenic viruses has proven valuable in the discovery of key cellular pathways that are rendered dysfunctional during cancer progression. Because of this, we studied human foreskin keratinocytes (HFK) immortalized with HPV in order to gain insight on the role of the lncmtRNAs in cell proliferation. We showed that immortalization of HFK with HPV-16 or 18 causes downregulation of the ASncmtRNAs and induces the expression of a new sense transcript termed SncmtRNA-2.

Transduction of HFK with both E6 and E7 oncoproteins is sufficient to induce expression of SncmtRNA-2. On the other hand, the E2 oncogene is involved in downregulation of the ASncmtRNAs. Knockdown of E2 in immortalized cells reestablishes in a reversible manner the expression of the ASncmtRNAs, suggesting that endogenous cellular factors(s) could play functions analogous to E2 during non-viral-induced oncogenesis [43]. Our results suggest that a fraction of SncmtRNA-1 is processed outside of the organelle, to give rise to SncmtRNA-2 and a 63-nt fragment released from the IR. In silico analysis of this sequence revealed that the 63-nt fragment is highly complementary to hsa-miR-620. Using the TargetScan algorithm (www.targetscan.com), we found >100 predictive targets for hsa-miR-620 [44]. An interesting example is the mRNA of promyelocytic leukemia (PML) protein, which is a core component of PML nuclear bodies found in tumor cells, important structures involved in HPV replication. Several reports indicate that the E6 and E7 oncoproteins are localized in these nuclear structures [45].

2.2. Differential expression of lncmtRNAs as a tool for cancer diagnostics

As mentioned above, the ASncmtRNAs are downregulated in tumor cell lines and cells in tumor biopsies, independently of the tissular origin of the tumor analyzed. Therefore, this differential expression might be used for screening of cancer cells.

Cervical cancer is the fourth most common cancer in women worldwide. In 2012, this disease accounted for 528,000 new cases and 266,000 deaths among females [46]. Cervical cancer is of slow progression and, according to histopathological studies, there are at least three well-defined stages preceding cervical squamous carcinoma, known as cervical intraepithelial neoplasia (CIN). These stages (CIN1, CIN2 and CIN3) correspond to the progressive invasion of the cervical epithelium from the basal cell layer to the surface of the squamous epithelium [47]. Therefore, detection of premalignant lesions is key to preventing disease progression to advanced stages.

Therefore, we performed a study in order to evaluate and quantify the differential expression of non-coding mitochondrial RNAs during the progression of the disease. We found down-regulation of the antisense mitochondrial transcripts at early stages of cervical neoplasia (CIN1). Moreover, differential expression of ASncmtRNA v/s S-ncmtRNA showed significant difference, while, as expected, normal proliferating tissues did not display down-regulation of ASncmtRNAs. Moreover, downregulation of ASncmtRNAs correlated with the over-expression of the tumor suppressor protein p16INK-4a [48, 49].

Bladder cancer (BC) is a significant cause of morbidity and mortality with a high recurrence rate. Early detection of bladder cancer is essential in order to remove the tumor, to preserve

the organ and to avoid metastasis. The "gold standard" in the detection of BC is cystoscopy. This examination, however, is unpleasant, time consuming, expensive and may result in infections and urethral damage [50]. In a pilot study, we analyzed the differential expression of SncmtRNA and ASncmtRNAs in cells isolated from voided urine from patients with bladder cancer as a noninvasive diagnostic assay. For this purpose, we developed a test based on a multiprobe mixture labeled with different fluorophores, which takes about 1 hour to complete. We examined the expression of these transcripts in cells isolated from urine of 24 patients with BC and 15 healthy donors. The samples from BC patients revealed expression of SncmtRNA and downregulation of the ASncmtRNAs. Exfoliated cells recovered from the urine of healthy donors did not express these mitochondrial transcripts. The differential expression of the SncmtRNA and the ASncmtRNAs in cells isolated from voided urine can be explored as a new non-invasive diagnostic test for bladder cancer [51].

2.3. Targeting antisense noncoding mitochondrial RNA: From bench to clinic

As mentioned before, we postulated that the ASncmtRNAs might function as a unique mitochondria-encoded tumor suppressor. Therefore, we tested whether ASncmtRNA knockdown (ASK for short) induces alteration of cancer cell function. We found, in several tumor cell lines, that knockdown of the low copy number of the ASncmtRNAs with antisense oligonucleotides (ASO) induces massive cancer cell death by apoptosis without affecting the viability of normal cells. Apoptosis is triggered or potentiated by a drastic reduction in levels of survivin, a member of the inhibitor of apoptosis (IAP) family that is overexpressed in virtually all human cancers. Down-regulation of survivin is at the post-transcriptional level and probably mediated by microRNAs generated by Dicer from the ASncmtRNAs after ASO-induced RNase H processing [52]. It is important to highlight that the ASO treatment is efficient in inducing knockdown of the ASncmtRNAs, despite the fact that it is well known that oligonucleotides are not able to enter mitochondria in vivo [53]. In consequence, the obvious question is how are these transcripts targeted by ASOs? We have demonstrated that in normal human kidneys, renal cell carcinoma, mouse testis and the murine melanoma cell line B16F10, SncmtRNA and the ASncmtRNAs exit the mitochondria and are found localized in the cytoplasm and in the nucleus [42]. Consequently, our results suggest that the functional role of these molecules lies outside the organelle. Besides cell viability, ASK also drastically reduces proliferative index, anchorage-independent growth capacity, migration and invasion [52]. Taken together, our results allow us to propose that downregulation of the ASncmtRNAs constitutes an Achilles' heel of cancer cells, suggesting that the ASncmtRNAs are promising targets for cancer therapy.

In consequence, the ultimate challenge is to translate these results to an in vivo preclinical scenario with immunocompetent mice. For this purpose, we first characterized the murine noncoding mitochondrial RNAs (mncmtRNAs), which display structures similar to the human counterparts, including long double-stranded regions arising from the presence of inverted repeats. Most remarkable however is the identical expression pattern of these transcripts in both species. The mASncmtRNAs, expressed in normal proliferating cells, are downregulated in mouse tumor cells. ASK with ASO targeted to the mASncmtRNAs induces apoptotic cell

death of the highly aggressive and metastatic murine melanoma cell line B16F10 *in vitro*, concomitantly with survivin downregulation [54].

We assessed the efficacy of the ASO treatment in vivo, using a B16F10 syngeneic model in C57BL6/J, where we applied a therapeutic approach similar to the clinical practice guidelines of melanoma: surgical resection of the lesion followed by systemic administration of ASO targeted to mASncmtRNAs (1560S). Remarkably, there was no visible sign of lung or liver metastasis at 120 days since the beginning of treatment with ASO, although one cannot discard the possibility of micro-metastasis [54]. In tail vein injection lung colonization assay, ASO treatment significantly reduced the number of metastatic nodules in the lungs, as well as their size [54]. Similar results were obtained in this model with a lentiviral delivery approach of therapeutic sequences. Transduction with lentiviral constructs targeted to the ASncmtRNAs induced apoptosis in murine B16F10 and human A375 melanoma cells in vitro and significantly retarded B16F10 primary tumor growth in vivo. ASK treatment drastically reduced the number of lung metastatic foci in a tail vein injection assay, compared to controls [55]. These results provide additional proof-of-concept for knockdown of ncmtRNAs for cancer therapy and altogether, our results suggest that ASncmtRNAs could be potent targets for melanoma therapy.

In the RenCa cell line, corresponding to murine renal adenocarcinoma, we showed that ASK in vitro induces apoptosis mediated by downregulation of survivin, Bcl-2 and BclxL, the latter 2 members of another family of anti-apoptotic factors [56]. ASK also induces detrimental effects on metastatic potential, such as downregulation of N-cadherin, P-cadherin and MMP9, further strengthening the potential of this strategy for renal cell carcinoma (RCC) therapy. Remarkably, our in vivo studies in a subcutaneous syngeneic model of RenCa cells in Balb/C mice show complete reversal of tumor growth [57]. Moreover, in an orthotopic assay of murine RCC induced by injection of RenCa cells into the subcapsular region of the kidney showed that all the control mice contained tumors of different size. In contrast, only one mouse treated with the therapeutic ASO exhibited a small tumor. Histological analysis of each lung showed that control mice contained several and large metastatic nodules. In contrast, only two lungs of mice treated with therapeutical ASO contained metastatic nodules, which were significantly fewer and smaller. Finally, direct metastasis assessment by tail vein injection of RenCa cells also showed a drastic reduction in lung metastatic nodules [57].

These pre-clinical results with the RenCa and B16F10 murine models establish proof-of-concept that the ASncmtRNAs constitute a potent and selective target to develop a treatment for different types of cancer and positions this approach as an attractive strategy ready for clinical testing.

In this respect, the USA Food and Drug Administration (FDA) approved an oligonucleotide directed to the human ASncmtRNAs Andes-1537) as IND for a Phase I Clinical Trial. This study, currently under way and close to completion at UCSF, California, USA, is a first-in-human, open-label, dose escalation and expansion, 2-part study to determine the safety, tolerability, and maximum tolerated dose of Andes-1537 for Injection in patients with advanced unresectable solid tumors that are refractory to standard therapy or for which no standard therapy is available (NCT02508441). The result of this trial will be very important in order to continue with the next phase to assess the antitumoral efficacy of this therapy in human cancer patients.

3. Conclusions

Downregulation of the ASncmtRNAs has been assessed in several tumor tissues, such as bladder, prostate, kidney, ovary, cervix and breast, among others [58]. The role of these transcripts in cell proliferation and tumorigenesis is not fully understood at present. However, downregulation of the ASncmtRNAs while maintaining the expression of SncmtRNA represents a universal characteristic of tumor cells. The strong inhibition of tumor growth, induction of apoptosis and even tumor remission after knockdown of these RNAs *in vivo* is an impressive phenomenon that constitutes a unique opportunity for the development of a targeted therapy against several types of cancer. Moreover, the fact that antisense therapy does not cause side effects constitutes an attribute not observed in other kinds of therapies and opens the door to the evaluation of this approach in different kinds of solid tumors.

The mechanism by which interference of these RNAs generates cell death, apoptosis and delay in metastasis is beyond the scope of this review. However, our preliminary evidence indicates that the double strand region of the antisense RNAs is a seed for generation of miRNAs, which could target mRNAs of several proteins involved in cell cycle control, cell survival, invasion and metastasis. Therefore, knockdown of these RNAs generates a pleiotropic effect that affects simultaneously several important pathways necessary throughout the whole tumorigenic process.

Mitochondrial long noncoding RNAs are novel actors in cancer metabolism and understanding the roles they fulfill in tumor cell biology will make it possible to select in the future novel targets for the development of new therapies that could be effective and of low toxicity for patients. Our target described here meets these two requirements and at present is being evaluated in a Phase I protocol soon to finish. Those results will give the necessary data to continue with the next phase and to evaluate more in depth the efficacy of our therapy.

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Conflict of interest

The authors declare no conflict of interest.

Nomenclature

ASO	antisense oligonucleotide
HFK	human foreskin keratinocytes
mtDNA	mitochondrial DNA

lncmtRNA	long noncoding mitochondrial ribonucleic acid
ncRNA	noncoding ribonucleic acid
SncmtRNA	sense noncoding mitochondrial ribonucleic acid
ASncmtRNA	antisense noncoding mitochondrial ribonucleic acid

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