Research Article

The role of the multifaceted long non-coding RNAs: A nuclear-cytosolic interplay to regulate hyaluronan metabolism

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Abstract

In the extracellular matrix (ECM), the glycosaminoglycan (GAG) hyaluronan (HA) has different physiological roles favouring hydration, elasticity and cell survival. Three different isoforms of HA synthases (HAS1, 2, and 3) are responsible for the production of HA. In several pathologies the upregulation of HAS enzymes leads to an abnormal HA accumulation causing cell dedifferentiation, proliferation and migration thus favouring cancer progression, fibrosis and vascular wall thickening. An intriguing new player in HAS2 gene expression regulation and HA production is the long non-coding RNA (lncRNA) hyaluronan synthase 2 antisense 1 (HAS2-AS1). A significant part of mammalian genomes corresponds to genes that transcribe lncRNAs; they can regulate gene expression through several mechanisms, being involved not only in maintaining the normal homeostasis of cells and tissues, but also in the onset and progression of different diseases, as demonstrated by the increasing number of studies published through the last decades. HAS2-AS1 is no exception: it can be localized both in the nucleus and in the cytosol, regulating cancer cells as well as vascular smooth muscle cells behaviour.

Introduction

Extracellular matrix (ECM) is a complex and dynamic three-dimensional macromolecular network which not only provides structural support to tissues, but also favours the activation of intracellular signalling pathways regulating cell differentiation, migration and proliferation [1,2]. Its main components are collagen and proteoglycans (PGs); the latter are made of a core protein and one or more polysaccharidic chains, known as glycosaminoglycans (GAGs) [3]. In physiological conditions, ECM is constantly remodelled and allows the diffusion of molecules, growth factors and nutrients. It is important to underline that being so essential for the maintenance of normal tissue homeostasis, even small alterations in ECM structure or changes to its components, are often associated with pathological conditions [4–7].

Among all the components of ECM, hyaluronic acid (HA) is undoubtedly one of the most abundant. Although being part of the GAG family, HA is the only one which is not bound to a PG core protein [8]: it is a linear GAG composed of repeating disaccharide units of D-glucuronic acid (GlcUA) and *N*-acetyl-D-glucosamine (GlcNAc), linked together by alternating β(1➔3) and β(1➔4) glycosidic bonds. According to a classical and simplistic view, HA was considered merely as a space filler molecule, being hydrophilic and so capable of swelling. However, much evidence demonstrated the important and active role that HA has not only in maintaining normal homeostasis, but also in the onset of several pathological processes like ageing, cancer, fibrosis, inflammation, diabetes, gastrointestinal and vascular diseases [8–15]. In mammals, it is synthesized by three multi-pass transmembrane isoenzymes, called hyaluronan synthetases

(HAS1, HAS2 and HAS3) [16], which extrude the elongating chain through the plasma membrane into the extracellular matrix [17,18]. Among all HA synthetases, HAS2 seems to play the leading role in HA synthesis thanks to its finely regulated catalytic properties, its high expression with respect to other HASes, and its critical role during heart development [19,20]. As HAS2 expression is critical for animal survival [20], its expression undergoes a tight regulation [21], that primarily involves the action of growth factors and cytokines, like transforming growth factor beta (TGF-β), insulin-like growth factor (IGF), fibroblast growth factor (FGF) and prostaglandins [22,23]. HAS2 synthesis is regulated by pro-inflammatory stimuli as well, such as oxidized low-density lipoprotein (ox-LDL) and tumour necrosis factor alpha (TNFα) [24,25], vitamin D [26] and salicylate [27]. Further, HAS2 undergoes some post-translational modifications, influencing its activity, proteasomal degradation [28,29], dimerization, ubiquitination [30] and subcellular localization [31]. Notably, in vascular endothelial cells HAS2 can be degraded via autophagy evoked by nutrient deprivation or mTOR inhibition [32].

HAS2 functioning and HA deposition are eventually influenced by nutrients and cytosolic substrates availability, specifically UDP-GlcNAc and UDP-GlcUA [33,34] which can have a critical role in diabetes and in cancers, two pathologies that are characterized by huge metabolic reprogramming. Interestingly, 4-methylubelliferone (4-MU) inhibits HA synthesis in experimental conditions, both in vitro and in vivo [35–37] by competing with UDP-GlcUA, giving HA synthesis a new putative therapeutic role against inflammation, autoimmunity, and cancer [38,39].

Unfortunately, 4-MU is not specific and inhibits HA synthesis irrespective of the HAS isoform involved [38] whereas many negative roles of HA are due to the specific alteration of HAS1, 2 or 3. In 2007, Koyama et al. demonstrated that the mechanism underlying enhanced growth of mammary tumours in MMTV-Neu transgenic mice was the over-production of HA by HAS2 enzyme [40].

Other than being a pan-HAS inhibitor, 4-MU may also, to a lesser extent, affect the synthesis of other GAG chains at high concentrations of 4-MU [38].

Recently in 2019, Nagy and colleagues tested the efficacy of 4-methylumbelliferyl glucuronide (4-MUG), a metabolite of 4-MU which was thought to limit 4-MU utility in systemically inhibiting HA synthesis. In the paper they demonstrated that 4-MUG can suppress HA synthesis independently of its conversion into 4-MU and without depletion of the HA precursor UDP-GlcUA. However, also this molecule is not specific for any HASs isoforms [41].

Moreover, long exposure to 4-MU has been shown to have some side effects, as described for atherosclerosis [42] and hepatocellular carcinoma [43].

As HA metabolism is mainly related to HAS2 activity, it is of critical importance to better understand its gene expression, transcriptional and translational events. Recently, a new player in HAS2 gene expression regulation and HA production has been shown to be the long non-coding RNA (lncRNA) hyaluronan synthase 2 antisense 1 (HAS2-AS1), which belongs to the class of natural antisense transcripts, that should be nowadays considered important in orchestrating diverse metabolic pathways and pathologies.

HAS2-AS1 lncRNA

In 2005, Chao and Spicer described for the first time the existence of HAS2-AS1, which they originally called HASNT (HA synthase 2 antisense). HAS2-AS1 is located on chromosome 8q24.13 and transcribed from the opposite strand of HAS2 gene locus [44]. Being a *cis*-encoded natural antisense RNA, HAS2-AS1 anneals to its sense transcript and displays at least partial complementarity.

HAS2-AS1 transcript consists of four exons, flanked by a consensus splice acceptor and donor sequences, that are distributed as follows in respect to HAS2 gene: exon 1 is encoded by sequence located within HAS2 intron 1, exon 2 is partially complementary to HAS2 exon 1 and finally, exon 3 and 4 of HAS2-AS1 are encoded by sequences located within the proximal promoter region for HAS2 [44]. The presence of an alternative splicing site inside exon 2 allows the generation of two splicing isoforms of different length, called long (257 nt) and short (174 nt) depending on their nucleotide extent (Fig. 1A). These two isoforms show perfect complementarity with a region starting about 70 bp from the presumed transcription initiation site of human HAS2, allowing HAS2 mRNA and HAS2-AS1 natural antisense to form an heteroduplex at cytoplasmic level which stabilises HAS2 transcript. In 2011, Michael and colleagues proved for the first time HAS2 mRNA/HAS2-AS1 physical interaction in proximal tubular epithelial cells [45].

Until now, little is known about the role of HAS2-AS1 in pathologic conditions and most of the literature focuses on cancer and vascular diseases, which is not surprising at all considering that HA and HAS2 play an essential role in tumour development and progression [7] as well as favouring vascular smooth muscle cells (SMCs) proliferation and migration to the neointima [21,23,46,47]. It should be considered that HAS2-AS1 effects appear to be different in dependence on the cell line, acting via stabilising or neutralizing HAS2 transcript [46,48]. As an example, overexpression of HAS2-AS1 in osteosarcoma cells reduces HAS2 transcripts [49], whereas its expression in oral squamous cell carcinoma is essential for HAS2 transcription and hypoxia-induced invasiveness [50].

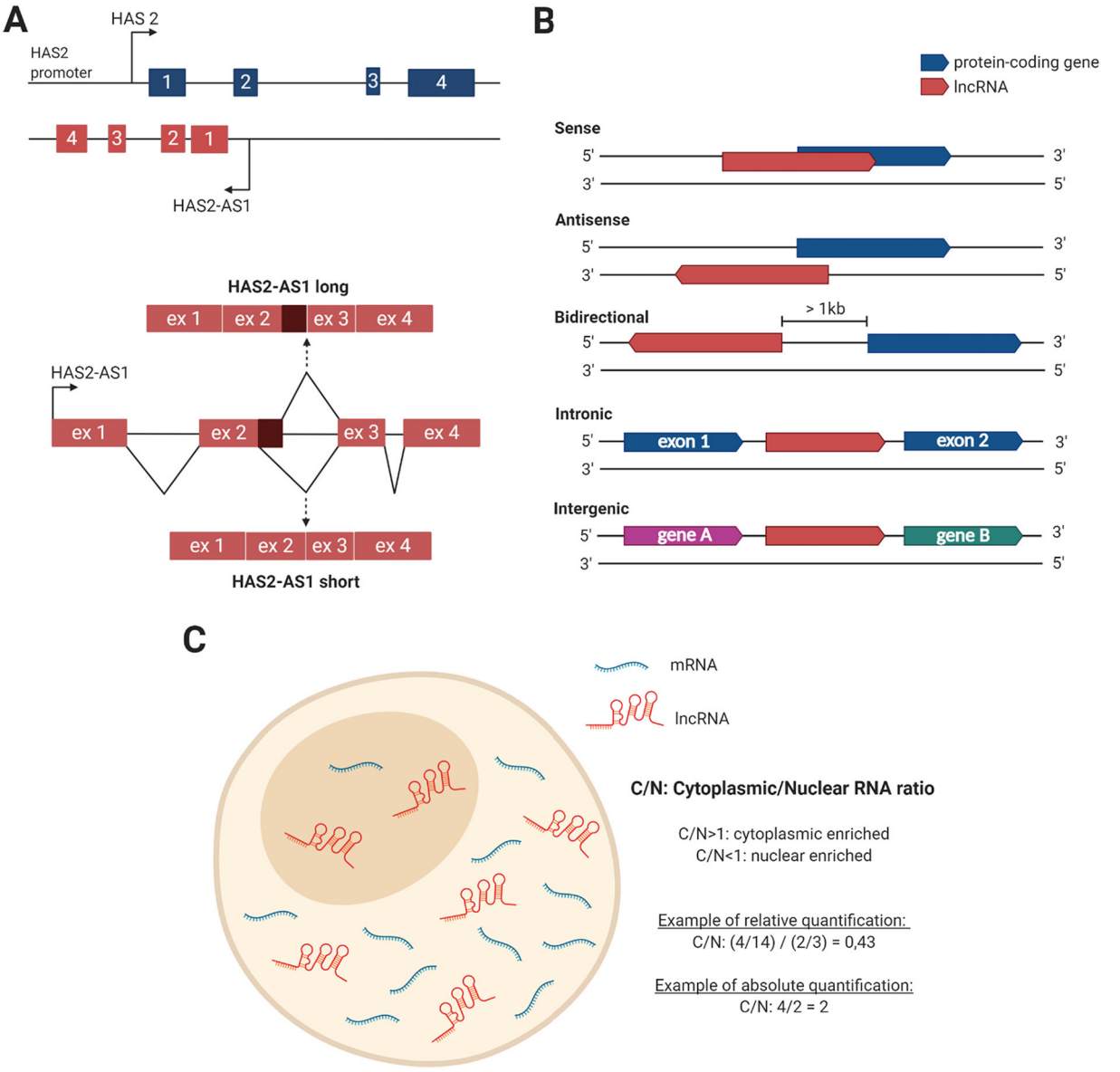

Fig. 1. A) Schematic representation of HAS2-AS1 genomic organization in respect to HAS2 gene (up) and of the two splicing isoforms of HAS2-AS1, long and short (down). B) Schematic representation of lncRNAs classification according to their genomic position in respect of a protein coding gene. C) Two different ways to quantify cytoplasmic/nuclear (C/N) localization of lncRNAs. The relative method estimates the ratio of concentrations in the two compartments; the absolute method calculates the ratio of molecules in the two compartments. The scheme is modified from Carlevaro-Fita and Johnson, 2019 [52].

Notably, HAS2-AS1 can also be exploited as a prognostic factor for clinical outcome in cerebral tumours. In fact, as demonstrated by Zhao and colleagues recently in 2019, HAS2-AS1 expression is higher in patients with advanced glioma or with a large tumour, correlating with shorter free-disease survival and overall survival time. In the same work, it was also demonstrated that HAS2-AS1 plays a role in regulating glioma cell viability, migration and invasion through the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway [51].

lncRNAs classification, function and localization

The transcriptional landscape of all organisms is more complex with respect to what was previously thought; even though approximately 80% of the mammalian genome is transcribed, only a small portion (about 2%) of this transcription include protein-coding potential [53–55]. The remaining non-translated portion of the genome has long been wrongly considered as “junk-DNA”. Instead, transcripts of this area represent a class of non-coding

RNAs, that can be clustered into two major groups depending on their size: small non-coding RNAs (e.g. microRNAs) with less than 200 bp, and lncRNAs with more than 200 bp up to ~100 kb [56,57].

lncRNAs have been described in all species including viruses, fungi, plants, prokaryotes and animals: their size and diversity in expression patterns correlates with organismal complexity [58] having several roles in regulation of gene expression [59–64].

lncRNAs share many features with mRNAs: they are both transcribed by polymerase II from genomic loci with similar chromatin state, they are often spliced, capped at the 5′ end and polyadenylated [65,66]. However, lncRNAs have limited coding potential, as they lack of significant and classical open reading frames, initiation codon, 3′-untranslated regions and termination codon; they mostly contain fewer but longer exons than mRNAs and have weak splicing and polyadenylation signals [55,67]. Generally, they also display modest sequence conservation: in respect to secondary and tertiary structures, lncRNAs primary sequence seems to play a less crucial role [68,69]. Last, in respect to mRNAs lncRNAs are less evolutionary conserved. Notably, even though the expression of lncRNAs is generally much lower than mRNAs, it is more tissue specific and their promoter sequences are under a great selective pressure, higher than that of their primary sequence[55,64,66,70,71].

Based on the relationship with the nearest protein-coding sequence, lncRNAs can be classified into five different categories: sense, antisense, bidirectional, intronic and intergenic.

Sense lncRNAs are located on the same strand as the protein-coding gene, contrary to antisense lncRNAs which instead originate from the antisense strand of a protein-coding gene; bidirectional lncRNAs are transcribed from a promoter of a protein-coding gene, yet in the opposite direction. Finally, intronic lncRNAs locate inside the introns of a protein-coding gene, while intergenic lncRNAs overlap within the intron of an annotate protein-coding gene [72–74] (Fig. 1B).

A great number of studies demonstrate that lncRNAs can regulate gene expression at different stages, starting from epigenetics and chromatin structure, transcriptional modifications, and even at translational and post-translation levels [74]. Notably, lncRNAs are involved in imprinting genomic loci, modifying chromosome conformation and regulating enzymatic activity [75]. Affecting these processes, they have a direct impact on the physiology of tissues and organs; thus, specific patterns of lncRNAs expression determine cell state, differentiation and development. Additionally, overexpression or deficiency in lncRNA expression is implicated in the onset of different diseases (e.g. cancer, and cardiovascular pathologies) [76–78].

Starting from pioneering studies on mouse brains and going through transcriptome-wide analyses, it has been demonstrated that lncRNAs in general show cell type-, tissue-, developmental stage- and disease state-specific expression patterns [79–81]. Further, many lncRNA species have defined subcellular localizations, being localized mainly in the nucleus and/or cytoplasm, but also in mitochondria and extracellular vesicles [52]. Notably, their function is strictly correlated with their subcellar fate. When evaluating lncRNAs abundance in subcellular compartments, an historical common miscalculation reports lncRNAs having a higher density in the nucleus rather than in cytoplasm [55,82]. This problem stems from the difficulty in estimating absolute transcript levels within cellular compartments. In fact, the relative localization of lncRNAs, which is estimated in respect to mRNA abundance (with a major occupancy in the cytoplasmic fraction) tends to be more nuclear in human cells, supporting the theory that the vast majority of lncRNAs are involved in epigenetic regulation, when exerting their function [65,83] (Fig. 1C). Despite this, when considering the absolute enrichment of lncRNAs in the nucleus vs the cytosol, is evident that more lncRNAs by transcript number are localized in the cytoplasm than in the nucleus [84]. In other words, thinking in terms of absolute number of molecules in a single cell, then the average lncRNA is mostly localized in cytoplasm in most cell lines (Fig. 1C).

lncRNAs subcellular localization can be affected by multiple factors, including RNA-protein complexes, environmental changes and even infections [85]. Even, some lncRNAs can be shifted to adjacent cells and circulate in serum through exosome trafficking [86,87].

Intriguingly, in the last years an increasing number of publications is describing that lncRNAs functioning is related to the synthesis and degradation of many components of the ECM [88–91], such as collagen [92,93], integrins [94,95], laminins [94], fibronectin [96], aggrecan [97], cadherins [96] and metalloproteases [98,99].

lncRNAs in the nucleus

In respect to mRNAs, lncRNAs are generally more nuclear localized, in part due to inefficient splicing and polyadenylation [100,101], and also because of their nuclear export inhibition resulting from the presence of *c*is elements that are associated with nuclear proteins (e.g. short C-rich sequences) [102,103]. Finally, lncRNAs with unconventional structures tend to accumulate in the nucleus [104,105]. Once in the nucleus, lncRNAs play a pivotal role in the regulation of gene transcription and the organization of nuclear domains. lncRNAs control the epigenetic state of genes, participate in transcriptional regulation, are involved in alternative splicing, and constitute subnuclear compartments [106].

After being transcribed, lncRNAs can either accumulate near their transcription site (in *cis*) where they can directly exert their functions or being moved toward target regions which could be

quite distant in respect to their transcription region (in *trans*). Once accumulated in their target regions, lncRNAs normally associate with chromatin, thus initiating a series of events that finally bring to chromatin remodelling, that could happen in *cis* or *trans*, in respect to their site of accumulation.

Some lncRNAs act as guides for chromatin-modifying complexes and nuclear proteins to specific loci to accomplish their effect; hence, these chromatin-modifying complexes, can activate or repress specific lncRNAs associated genes [106]. A classic and elegant example of "recruitment model" for chromatin architecture regulation is that orchestrated by X-inactive-specific transcript (Xist), which drives widespread transcriptional silencing of one X-chromosome in females during embryogenesis, recruiting chromatin-modulating proteins to the future inactive X-chromosome (Xi) [107,108].

On the other hand, many other nuclear localized lncRNAs can act as decoys, preventing the interaction of histone or chromatin modifiers to specific DNA loci [108].

In addition to epigenetic modifications, lncRNAs can directly regulate transcription by generating R-loop configurations (triple-stranded structures resulting from RNA-DNA hybridization) which are able either to recruit transcription factors *in cis* around promoter regions or can interfere with Polymerase II transcription machinery during initiation or elongation processes [108].

Finally, lncRNAs are involved in the regulation of the integrity and functions of nuclear bodies (membraneless RNA-protein complexes), altering gene expression at post-transcriptional level [108,109] (Fig. 2A).

As regards HAS2-AS1, it can be located either in the nucleus or in the cytoplasm [110]. In 2014 Vigetti et al., demonstrated that in SMCs HAS2-AS1 is an important nuclear epigenetic regulator of HAS2 gene, being crucial to induce HAS2 expression *in cis* facilitating chromatin opening around HAS2 promoter [46]. This regulatory step involves the addition of a single GlcNAc residue (O-GlcNAcylation) to p65 nuclear factor κ–light-chain enhancer of activated B cell (NF-κB) subunit, which, in turn, modulates HAS2-AS1 promoter [46]. Moreover, this NF-κB/HAS2-AS1/HAS2 axis can also be regulated by sirtuins 1 (SIRT1) activity and pro-inflammatory stimuli [25] (Fig. 2B). Sirtuins, and specifically SIRT1 is a NAD^+ deacetylase having multiple roles in chromatin remodelling, cell ageing, energy metabolism, stress response and apoptosis [111]. In general, sirtuins are sensitive to cytoplasmic fluctuations of NAD^+ – which is strictly dependent on the nutritional state of cells [33]. Among the most attractive characteristics of this molecule, is its involvement in the protection from cellular senescence, promotion of DNA repair [112] and its contribution in extending organismal lifespan in several animal models, including among all also mice [113–116]. These longevity effects are the result of the interaction of SIRT1 with important metabolic/longevity related pathways, including AMP-activated protein kinase (AMPK), insulin/IGF-1 signalling (IIS), target of rapamycin (TOR) and forkhead box O (FOXO). In vitro experiments conducted by our group, demonstrated for the first time that SIRT1 can prevent inflammation (triggered for example by TNFα) through inhibition of HA metabolism: the activation of SIRT1 prevents NF-κB/p65 nuclear translocation and decreases the levels of HAS2-AS1, which in turn regulates HAS2 and HA deposition. The result is a decreased SMCs migration and monocyte recruitment [25]. As during first stages of atherosclerosis increased HA deposition is an important factor contributing to SMCs migration, ECM deposition, vessel walls thickening ad macrophage recruitment and polarization, the regulation of SIRT1/HAS2-AS1/HAS2 pathway may be pivotal in the treatment of this pathology.

Apart from vascular diseases, HAS2-AS1 is involved in cancer progression. Tumours are generally characterized by a high rate proliferation, allowing tumour mass to develop faster than vasculature; this results in the formation of a tumour niche, which is depleted of vascularization and deficient in oxygen (hypoxia) [117]. Hypoxia is one of the events promoting cellular adaptations during cancer progression, including a switch to anaerobic metabolism, increased genetic instability, promotion of angiogenesis, activation of invasive growth, epithelial to mesenchymal transition (EMT), chemoresistance and preservation of stemness [118]. The major events mediating all these adaptive responses to hypoxia are the stabilization and activation of the hypoxia-inducible factors (HIFs), especially HIF-1α [118,119]. Interestingly, microarray analysis performed comparing oral squamous cell carcinomas in normoxic versus hypoxic conditions, showed an aberrant expression of HAS2-AS1 in the second condition – thus suggesting a connection between HAS2-AS1 and hypoxia. Intriguingly, HA production and HAS2 expression can be both stimulated under hypoxic conditions, as HAS2-AS1 promoter contains a hypoxia-responsive element (HRE) that responds to HIF-1α, finally contributing to EMT [119]. Similarly to HIF-1α and NF-κB, HAS2-AS1 transcription is tightly regulated by other transcription factors, such as Sp1, Sp3, High-mobility group AT-hook 2 (Hmga2), Signal transducer and activator of transcription 1 (STAT1) and CAMP responsive element binding protein 1 (CREB1) [110,120–122]. Not surprising, Sun et al. reported another nuclear mechanism of action of HAS2-AS1 in non-small cell lung cancer: the lncRNA can recruit lysine-specific demethylase 1 (LSD1) to EPHB3 promoter region, yielding its inhibition [123] (Fig. 2B).

lncRNAs in the cytoplasm

A huge number of lncRNAs need to be exported to the cytoplasm to exercise their regulatory roles. Cytoplasmic lncRNAs can govern important events which are essential for the maintenance of

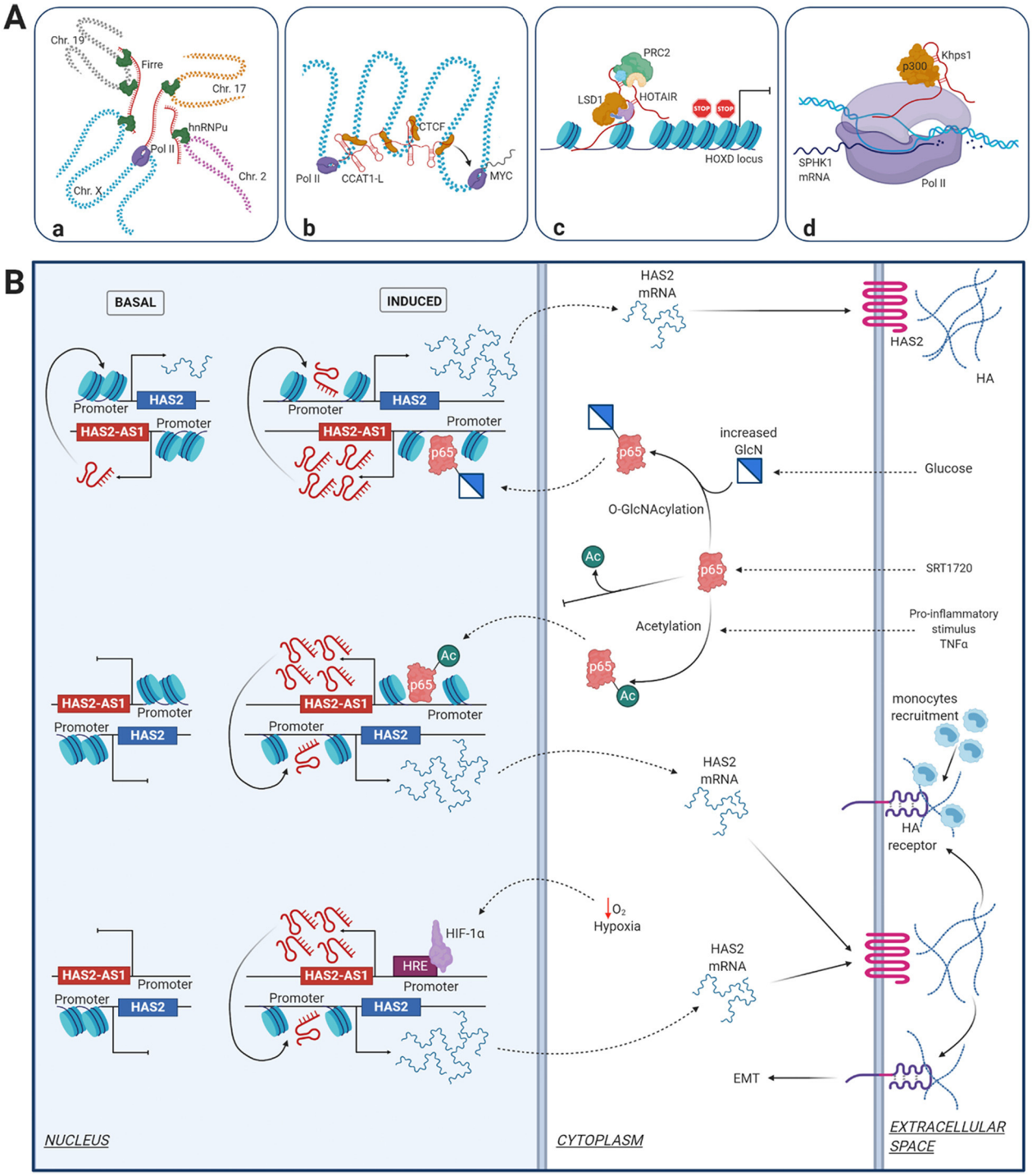

Fig. 2. A) Examples of general lncRNAs nuclear functions. a) *TRANS* CHROMATIN REMODELLING. Firre transcripts localize to distant autosomal chromosomal loci in trans to affect interactions between distant genomic regions. b) *CIS* CHROMATIN REMODELLING. CCAT1-L accumulates in cis to modulate chromatin loops between enhancers and the promoter of MYC. c) DECOY. HOTAIR mediates gene silencing of HOXD locus, through recruitment and binding of the LSD1 and PRC2 complex to the HOXD locus. d) R-LOOP. Khps1 enhances Pol II transcription by forming an R-loop that anchors Khps1-interacting p300 to the SPHK1 promoter. B) Schematic illustration of HAS2-AS1 functions in the nucleus.

cellular structure and functions [124,125]. Increasing evidence show that lncRNAs have a role in mRNA stability, can promote or inhibit target mRNAs translation, function as micro-RNA (miRNA) precursors, compete for miRNA-mediated inhibition, control protein localization and

turnover and the availability of cytoplasmic factors, scaffold proteins operating in shared pathways and, finally, modulate post-translational modifications (PTMs) [106,126].

An interesting function of lncRNAs is their ability to regulate mRNA translation, mainly through the binding of lncRNAs to mRNAs leading the recruitment of specific RNA-binding proteins (RBPs) that can either initiate or repress translation [126]. Recently, it has been found that about 70% of the cytoplasmic fraction of lncRNAs is associated with ribosomes, meaning that lncRNAs are involved

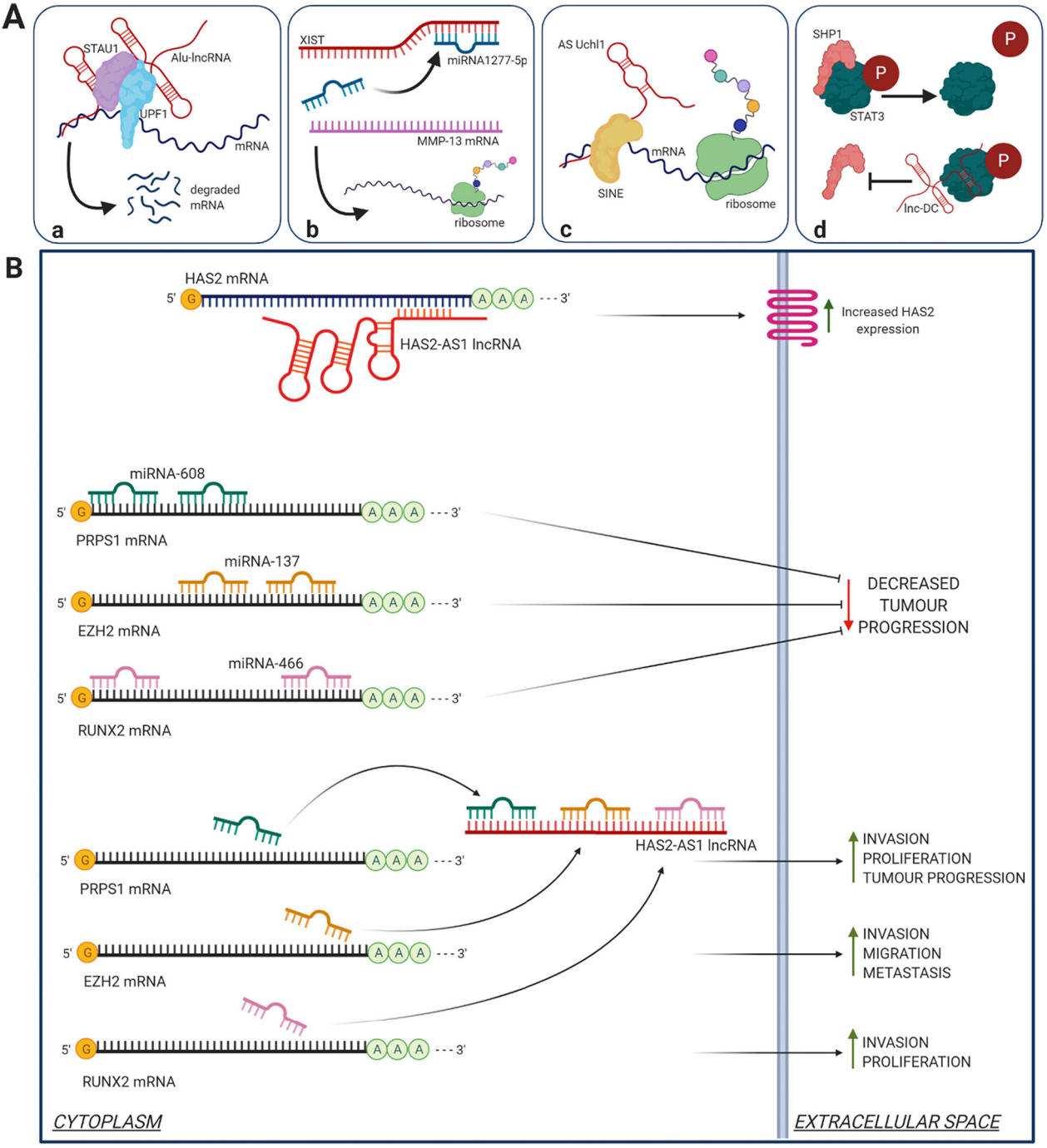

Fig. 3. A) Examples of general lncRNAs cytoplasmic functions. a) RBPs DECOY. Alu-containing lncRNAs associate with mRNA 3′-UTRs and recruit STAU1 to induce mRNA decay through UPF1 recruitment. b) ceRNAs. XIST promotes MMP-13 expression by sponging miRNA1277-5p. c) REGULATION OF TRANSLATION VIA RBPs. AS Uchl1 recruits SINE RBP, stabilising target mRNA thus prompting its translation into protein. d) PTMs REGULATION. Lnc-DC directly interacts with STAT3 to prevent its dephosphorylation by SHP1. B) Schematic representation of HAS2-AS1 functioning in the cytosol.

in enhancing the formation of active polysome, thus prompting mRNA translation into proteins [127]. In some instances, when associated with ribosomes, lncRNAs can also function as sources for small new peptides [128]. mRNA stability is influenced by the action of lncRNAs also at a post-transcriptional level, first via associated miRNAs: in some cases, miRNAs are essential for lncRNAs to orchestrate gene expression. RNAs can crosstalk with each other by competing for shared miRNAs; the main actors in this regulative circuit are lncRNAs called competitive endogenous RNAs (ceRNAs) which work as miRNA sponges, regulating the distribution of miRNAs on their target mRNAs, thereby derepressing miRNA targets and imposing an additional level of posttranscriptional regulation [129–131].

In order to block the action of miRNAs on their target mRNAs, lncRNAs can also form sense–antisense RNA duplex preventing miRNA-induced repression of target mRNAs by masking the binding site for miRNAs, thereby increasing the stability of the mRNA itself [132].

Other lncRNAs can modulate gene expression post-transcriptionally by recruiting mRNA degrading proteins, as demonstrated by a group of lncRNAs that include *Alu* elements [133]. On the other hand, some lncRNAs can function as molecular decoys, sequestrating RBPs involved in mRNA degradation, thus increasing mRNA stability [134,135].

Lastly, lncRNAs can also regulate gene expression at a post-translational level, interfering with PTMs by masking binding sites for PTM enzymes or PTM sites [136] (Fig. 3A).

In addition to the nuclear function of HAS2-AS1 as an epigenetic regulator, some groups demonstrated its ability to interact with other RNA species (e.g., miRNAs) in the cytoplasm.

First of all, as demonstrated by Michael and colleagues in 2011, in renal proximal tubular epithelial cells (PTCs), interlukine-1 beta (IL-1β) and transforming growth factor-beta 1 (TGF-β1) induce coordinate expression of HAS2 and HAS2-AS1, which can interact at cytoplasmic level by forming an RNA-RNA heteroduplex, finally stabilising and prompting HAS2 expression in PTCs [121].

One of the most appealing features of lncRNAs is their ability to function as ceRNAs for miRNAs, and HAS2-AS1 is no exception to this. The literature regarding HAS2-AS1 ability to bind miRNAs is expanding, reporting mainly HAS2-AS1/miRNAs interaction which are functional in cancer.

HAS2-AS1 is reported to be extremely upregulated in glioma, and the overall survival rate of patients with high HAS2-AS1 expression is significantly lower than that of patients with low HAS2-AS1 expression, suggesting that HAS2-AS1 might play a role in glioma by acting as an oncogene [137].

Zhang and colleagues in 2019 demonstrated that HAS2 natural antisense works as an oncogene in gliomas sponging miRNA-608, thus adjusting the expression levels of miRNA-608 target mRNA Phosphoribosyl Pyrophosphate Synthetase 1 (PRPS1) and finally increasing invasion and proliferation of glioblastoma multiforme (GBM) cells [121]. A similar study reports that in GBM cells HAS2-AS1 can regulate Enhancer of zeste homolog 2 (EZH2) mRNA expression by sponging miRNA-137, hence promoting migration and invasion of glioma cells and suggesting a possible molecular mechanism underlying glioma metastasis [137]. The group of Sun et al. gave another example of HAS2-AS1 sponge function, proving that its interaction with miRNA-466 relives Runt-related transcription factor 2 (RUNX2) function, yielding to an increased ovarian cancer cell proliferation and invasion [122] (Fig. 3B).

Concluding remarks

Although HAS2-AS1 has been ignored for long time, it is now clear that this epigenetic regulator is essential not only in maintaining normal homeostasis, being finely modulated by diverse physiological stimuli such as inflammation and nutrients availability via O-GlcNAcylation; HAS2-AS1 is also a pivotal factor in controlling pathological condition as it is an important stimulator for tumour cell proliferation and migration via, among all, HIF-1α and acting as a ceRNA.

Notably, as miRNAs landscape is very different depending on tissues and cells, it would not be surprising that the effects of HAS2-AS1 could be specific and restricted to a particular cell type or cancer histotype, making this natural antisense transcript a potential pharmacological target to modulate HAS2 expression without altering the other HASes.

CRediT authorship contribution statement

Conceptualization (D.V.); Original Draft writing (Ar·P, I.C.); Writing - Review & Editing (P.M.); Funding acquisition (E.K., M.V., A.P., D.V.). All authors red and approved the final manuscript.

DECLARATION OF COMPETING INTEREST

The authors declare no conflict of interest with the content of the present review.

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Abbreviations:
HA, hyaluronan; ECM, extracellular matrix; GAG, glycosaminoglycans; PG, proteoglycan; HAS2, hyaluronan synthase 2; HAS2–AS1, hyaluronan synthase 2 natural antisense 1; lncRNA, long non-coding RNA; SMCs, smooth muscle cells; NF-κB, nuclear factor κ–light-chain enhancer of activated B cell; HIFs, hypoxia-inducible factors; UDP-GlcUA, UDP-glucuronic acid; UDP-GlcNAc, UDP-*N*-acetylglucosamine; SIRT1, sirtuin 1; TNF-α, tumour necrosis factor alpha; 4-MU, 4-methylubelliferone; 4-MUG, 4-methylumbelliferyl glucuronide; EMT, epithelial to mesenchymal transition; miRNA, micro-RNA; PTM, post-translational modification; RBP, RNA-binding protein; ceRNA, competitive endogenous RNA

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