Non-coding RNA regulatory networks in mesothelioma: a narrative review of their implication in innate immune signaling pathways

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Abstract: Malignant mesothelioma is a rare but rapidly fatal disease highly enriched for innate immunity signature in a subset of patients with better clinical outcome. We provide here an overview of current knowledge on RNA regulatory networks altered in mesothelioma and resulting in increased expression of non-coding-RNA ligands stimulating the innate immune system. The focus on non-coding RNA (ncRNA) ligands is dictated by the fact that a large fraction of the non-coding genome is known to be transcribed and forms duplex RNAs able to stimulate anti-viral defense. Hence, we mostly describe double-stranded RNA sensors such as cytosolic RIG-I-like receptors and endosomal leucine-rich receptors Toll-like receptors 3 and their endogenous ligands. The latter include products downstream alteration of alternative splicing, altered RNA processing or expression of repetitive elements induced by demethylation events or deficiency of chromatin remodelers such as histone methyltransferase SETDB1 or histone demethylase KDM1A/LSD1 or inhibition of CDK4/6. Based on knowledge acquired either in experimental pre-clinical models or in clinical trials in other cancers types, all these events are likely to influence the outcome of mesothelioma patients treatment with new modalities which are explored in current mesothelioma clinical trials. Furthermore, immune checkpoint inhibition became recently standard of care in unresectable mesothelioma.

Keywords: Mesothelioma; double-stranded RNA; innate immunity

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Introduction

Malignant mesothelioma [reviewed in (1) and (2)] is a rapidly fatal tumour arising in mesothelium, which has mesodermal origins and covers many of the important internal organs like the lungs (pleural mesothelioma), peritoneal cavities (peritoneal mesothelioma), the sacs surrounding the heart (pericardial mesothelioma) and the testis (tunica vaginalis mesothelioma). Although mesothelioma is a rare cancer, its incidence is still rising. Since the seminal experiments of Wagner (3), exposure to asbestos has been clearly identified as a cause of mesothelioma. It is estimated that 125 million people worldwide have a history of asbestos exposure. Although the use of asbestos has been banned in several countries, there are several developing nations that continue to use asbestos (1). In addition, asbestos has a very long latency period. The duration of time between exposure to asbestos and the incidence of disease is approximately 40 years (4). This means that the incidence of mesothelioma will continue to rise in the years to come.

Frequently alterations in clinical samples [reviewed in (1) and (2)] include loss of function of tumor suppressors common to several cancers such as *CDKN2A* and *CDKN2B*, but also in alterations in the NF2/Hippo signaling pathway converging on the activation of oncogenic Yes-associated protein 1 (YAP1) transcription co-activator, and in BRCA-associated protein 1 (BAP1). Less frequent alterations include mutations in *TP53*, in *TERT* promoter and in genes involved in RNA metabolism. Interestingly, recent studies reporting frequent somatic mutations in epigenetic and splicing regulators (5,6) suggest that these alterations may represent a novel hallmark of cancer (5,6).

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Several of the alterations observed in mesothelioma, as we shall discuss in this review, are overall linked to RNA metabolism and may have an effect beyond tumor cells behavior because they interfere with microenvironment.

Interestingly, since 2012 we know that 75% of the human genome is transcribed into RNAs, while only 2% of these transcripts are translated into proteins (7). Therefore, 98% of the transcripts are not translated into proteins, while the contrary is observed in bacteria. The discovery that a large part of what had been called "junk DNA," is actively transcribed and carries out crucial functions inspired the concept of the "RNA networks" (8), where RNA are the most influencing molecules in cellular function in eukaryotes, contrary to the view of protein-centered networks, which was assumed according to the knowledge acquired in prokaryotes.

It is very likely that a vast majority of non-coding transcripts adopts complex 3D structure(s) to achieve their biological functions. These "structured" RNAs act using very diverse mechanisms including RNA-RNA, RNA-ligand, RNA-protein, RNA-DNA, and RNA-substrate interactions (9). Structured RNAs are critical components of key molecular machines in the cell, such as the spliceosome, ribosome, and telomerase, and RNA structures play important roles in the control not only of mRNA but also noncoding RNA functions (10).

RNA structures have different organization levels: the first one consists of the nucleotide sequence folding on itself via Watson–Crick base-pairing to form secondary structure elements (e.g., hairpins) and unpaired regions. *In vitro* studies have demonstrated that it can be modified by the amount of magnesium chloride (11). RNA structures are highly dynamic and modulated by binding to partners, which adds another degree of complexity to these structures.

A recent genome-wide RNA–RNA crosslinking study (12) using PARIS (psoralen analysis of RNA interactions and structures) identified a large number and diversity of RNA duplexes. 25% of the aligned double-strand helix-forming sequences were found to be conserved between mammals, birds and reptiles, suggesting a biological function. Indeed, the evolutionary conservation of RNA secondary structure across several species is a strong indicator of function (13).

A large amount of structured duplex RNA present and conserved in non-coding RNA (ncRNA) is an additional argument for sections that will be discussed below on duplex RNA being part of the pattern recognized by the innate immune system. Across many common human cancers, a large proportion of tumors unexpectedly express high levels of interferon (IFN)-stimulated genes (ISGs, e.g., *IFT1-3*, *IFITM1*, *DDX58*, *IFIH1*) that are typically associated with anti-viral signaling (14). However, given that cancer-associated anti-viral signaling is occurring in a sterile microenvironment, this raises questions on the nature of the endogenous RNA that activates the signaling and the extent to which it influences the multitude of effects that stromal cells exert on cancer progression and therapy response.

In this review, we shall focus on ncRNA and immune signaling pathways in mesothelioma.

We present the following article in accordance with the Narrative Review Reporting Checklist (available at http://dx.doi.org/10.21037/pcm-21-4).

Endogenous ncRNAs activating and regulating innate immunity in mesothelioma

Endogenous ncRNA can activate the innate immunity after being recognized by RNA sensors (15). RNA sensors are classified into two families based on their structural motifs, leucine-rich repeat (LRR) and DExD/Hbox helicase. Nucleic acid-sensing in cancer is associated with cytosolic DExD/Hbox helicases (RIG-I-like receptors: RLR) or endosomal leucine-rich receptors (Toll-like receptors: TLR) sensors.

Cytosolic sensors and endogenous ncRNA ligands and regulators

In the cytosol, there are two nucleic acids sensing systems: the cGAS–STING pathway for the recognition of DNA (16), and the RLRs for the recognition of RNA species (17).

In this review dedicated to ncRNA we focus on RLRs. The latter include retinoic acid-inducible gene I (RIG-I, encoded by *DDX58* gene), melanoma differentiation-associated protein 5 (MDA5, encoded by *IFIH1* gene) and laboratory of genetics and physiology 2 (LGP2, encoded by *DHX58* gene). RIG-1 and MDA5, but not LGP2, possess a caspase activation recruitment domain (CARD) necessary for downstream signaling. Oligomerization of RIG-I and MDA5 drive their association with their common adapter mitochondrial antiviral signaling protein (MAVS) (*Figure 1*).

Activation of MAVS results in stimulation of the kinases TANK-binding kinase 1 (TBK1) and IκB kinase ε, which phosphorylate MAVS, then transcription factors interferon regulatory factor 3 and 7 (IRF3 and 7, respectively), and NF-κB. In the context of mesothelioma it is worth noting that YAP activity results in inhibition of TBK1

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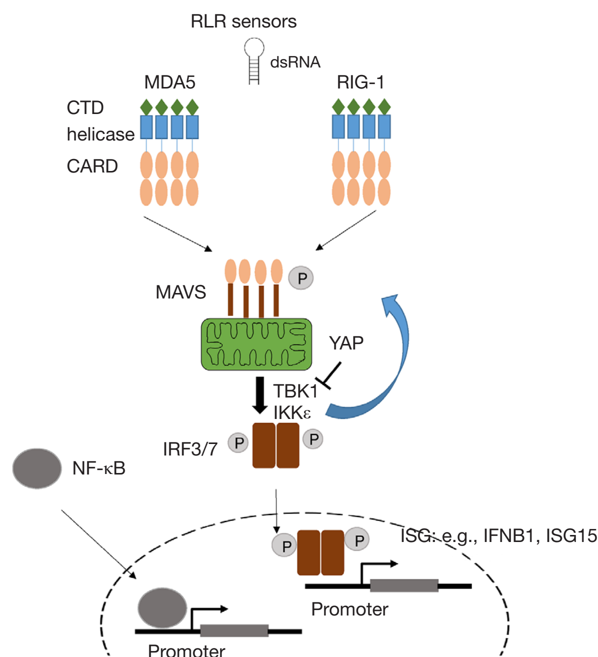

Figure 1 Interference of YAP with dsRNA-dependent IFN-inducing pathways downstream cytosolic sensors. dsRNA binds the helicases domain of MDA5 or RIG-1, driving their oligomerization through CARD, activating MAVS. Activation of MAVS results in stimulation of the TBK1 and IKK ε, which phosphorylate MAVS, then IRF-3, IRF-7 and NF-κB. YAP, inhibits TBK1. Phosphorylation of IRF3 leads to conformational changes and rearrangement of IRF3 monomers to dimers. Dimeric IRF3 then translocates to the nucleus to bind to IRF binding elements for the induction interferon-stimulated genes (ISG). YAP, Yes-associated protein 1; CTD, carboxy-terminal domain; ds, double-stranded; MDA5, melanoma differentiation-associated protein 5; RIG-1, retinoic acid-inducible gene I; CARD, caspase activation recruitment domain; MAVS, mitochondrial antiviral signaling protein; TBK1, TANK-binding kinase 1; IKK ε, IκB kinase-ε; IRF, interferon regulatory factor.

activation (18). This may represent a negative feed-back loop because dsRNA-signaling can activate YAP activity through the activation of IRF3 (19). Importantly IRF3 has been shown to act as YAP agonist and IRF3 depletion results in the suppression of YAP-driven growth (19), at least in gastric cancer. These data are consistent with our observation that dsRNA signaling is activated (20) during mesothelioma development in asbestos-exposed mice where we had previously described YAP activation (21). Altogether, this suggests that IRF3 could be a new therapeutic target against YAP-driven mesothelioma. As we mentioned above, YAP activation is controlled by the NF2/Hippo pathway but recent work has also conferred this property to BAP1 (22), at least in pancreatic cancer. Therefore, control of YAP signaling has an influence not only on tumor cell proliferation but also shuts-down a signaling affecting immune signaling. Indeed, phosphorylation of IRF3 leads to conformational changes and rearrangement of IRF3 monomers to dimers. Dimeric IRF3 then translocates to the nucleus to bind to IRF binding elements for the induction of interferon-stimulated genes (ISG), such as *IFNB1*, *ISG15* (*Figure 1*) (23). As mentioned above, among the most frequent genomic alterations in pleural mesothelioma are the homozygous deletions (HD) of the CDKN2A tumor suppressor gene. CDKN2A HD are accompanied in 30% of the cases by the HD of all genes encoding type I interferons (IFN I) which lie nearby in the chromosome 9 (24), indicating an additional interference with interferon signaling.

LGP2 is unable to interact with MAVS (25) and has been found to have opposite effects on RIG-I and MDA5. Indeed, LGP2 downregulates RIG-I's signaling activity, while it upregulates MDA5 signaling (26).

Although knowledge about these sensors had been acquired because they are essential in response to viruses, which form dsRNA during their replication, they can be stimulated by endogenously formed dsRNA, in the absence of mechanisms allowing the distinction between self and non-self nucleic acids. Indeed, RLRs utilize multiple criteria to ensure selective recognition of non-self RNA and robust discrimination against cellular RNA. For both RIG-I and MDA5, duplex RNA structure is necessary but not sufficient for the sensing of foreign RNA.

Endogenous RIG-1 ligands

For the activation of RIG-I, a 5' triphosphate group (5'ppp), which is present in all nascent transcripts and unprocessed viral RNAs, is additionally required (27-29). Polymerase III transcription is the main source of endogenous 5'ppp RNA in the absence of viral infection (30). Polymerase III activity is augmented by MYC (31) and by nearby RNA polymerase II occupancy (32).

Most cytosolic mRNAs do not have a 5' triphosphate group because they contain both a 7-methyl guanosine cap (cap 0) and 2'-O methylation (cap 1). Cap 0 protects the molecule from 5' to 3' exonuclease cleavage and is essential for the regulation of gene expression, including splicing, nuclear export of mRNA,

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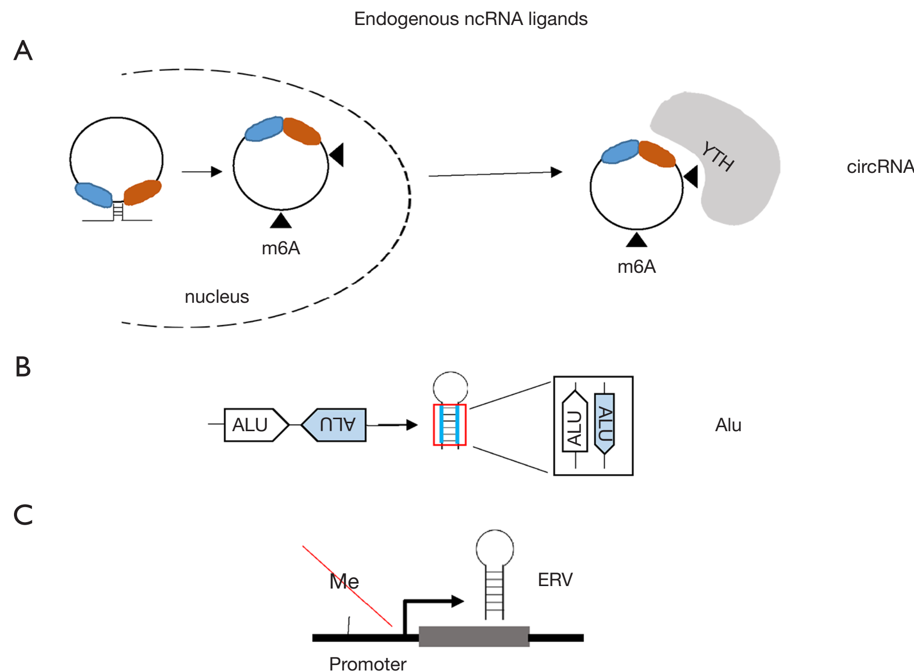

Figure 2 RNA able to form dsRNA. (A) circRNAs are overexpressed in mesothelioma. They are formed by back splicing and are normally recognized as self through N6-methyladenosine which is recognized by YTH-domain containing RNA binding proteins. In the absence of N6-methyladenosine they activate RIG-I. (B) Alu repetitive sequences makeup 11% of the human genome and upon expression can be found in two-strand directions + and –, which allows them to bind the complementary sequence and form dsRNA structures. (C) Repetitive elements include ERV which may be expressed upon promoter demethylation and form dsRNA. ERV, endogenous retroviruses; ds, double-stranded.

and translation initiation (33,34). Cap 1, is dependent on the activity of a cap1 methyltransferase 1 (CMtr1), encoded by a gene induced by type 1 IFN. 2'-O methylation is required to avoid stimulation of RIG-1. Downregulation of CMtr1 results in RIG1-dependent increased type 1 IFN activation (35). Additionally, a dsRNA length of >19 bp and blunt end is sufficient to activate RIG-1 (27).

Circular RNAs (circRNA) are recently described sequences of RNA formed by back-splicing especially in the presence of Alu repeats and complementary sequences around exons (36,37). Almost 300 circRNAs have been reported to be upregulated when mesothelioma were compared to normal mesothelial cells (38). CircRNA can stimulate RIG-I and host circRNAs normally evade recognition by RIG-I through N6-methyladenosine modification, which is recognized as endogenous RNA via N6-methyladenosine readers of YT521-B homology (YTH) family (39) (*Figure 2A*). Recent studies have demonstrated that inhibition of N6-methyladenosine readers increases the activity of immune checkpoint inhibitors (40). This is important since *YTHDF1* is significantly enriched in sarcomatoid tumors while *WTHDF2* is enriched in epithelioid tumors (41).

RNA processing defect leads to the accumulation of potentially immunogenic aberrant RNA transcripts. Cytoplasmic mRNA turnover is initiated by poly(A) tail removal and proceeds via two mechanisms, including 3'-5' exoribonucleolysis by the so called RNA exosome (42). Cytoplasmic RNA exosome activity requires the Ski complex, comprising the scaffold Ski3 (*TTC37*), two copies of Ski8 (*WDR61*), and the helicase Ski2 (*SKIV2L*). Recently, in our own experimental model of mesothelioma development (21) in mice exposed to asbestos, we observed a significant (P=7.21E-07, FDR =2.18E-06) 1.4-fold increase *Skiv2l* in mesothelioma tumors when compared to inflamed tissue, indicating an increase in RNA processing activity. Interestingly, *SKIV2L* expression is significantly higher in epithelioid compared to tissues with a sarcomatoid molecular profile (41). Serine/threonine-protein kinase/endoribonuclease inositol-requiring enzyme 1 α (IRE1α)

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(encoded by *ERN1*) is activated during the unfolded protein response (UPR), responsible for the cleavage of the precursor of XBP1 mRNA allowing the generation of the functional XBP1 necessary for the transcription of UPR genes. Interestingly, a basal UPR signaling has been observed in mesothelioma cells grown in 2D conditions and a low UPR signaling has been associated with chemotherapy resistance (43). IRE1α also generates RIG-I ligands that are normally degraded by the SKIV2L RNA exosome, thereby increasing the basal signaling activity of RIG-I in SKIV2L deficient cells (44). In the mesothelioma development model mentioned above (21) we observed a significant (P=0.00004514, FDR =0.0001064) 2-fold increase of *Ern1* in mesothelioma tumors when compared to inflamed tissue, and, like for *SKIV2L*, ERN1 expression is significantly higher in epithelioid compared to tissues with a sarcomatoid molecular profile (41). Altogether, these observations indicate that additional ways are activated, at least in epithelial mesothelioma, to shut down interferon signaling.

Finally, depletion of the human silencing hub (HUSH) complex, which includes MPHOSPH8/MPP8, periphilin (PPHLN1) results in activation of long-interspersed nuclear elements (LINEs) repetitive elements and activation of both, RIG-I and MDA-5 (45).

MDA-5 endogenous ncRNA ligands

MDA5 does not need 5'ppp. Instead, MDA5 has more-stringent criteria for dsRNA length (>0.5–1 kb) and dsRNA complementarity of the two strands in the selective recognition of foreign dsRNA and discrimination against shorter and imperfect cellular dsRNAs. dsRNA may be present in transcripts containing repetitive elements (46) (*Figure 2B*). Alu are part of repetitive elements found in the human genome. There are four main types of repetitive elements: LINEs, short-interspersed nuclear elements (SINEs), Retrovirus-like elements such as endogenous retroviruses (ERV) and DNA transposon fossils (47). Altogether they cover two-third of the human genome (48). Taken together with the information that 75% of the genome is transcribed, a large part of the transcriptome corresponds to repetitive elements. Alu elements are the most important subgroup of the SINEs. They makeup 11% of the human genome and can be found in two-strand directions + and –, which allows them to bind the complementary sequence and form dsRNA structures (*Figure 2B*). Endogenous dsRNA formed by inverted Alu repeats, in the absence of viral infection constitute about 67% of dsRNA bound to MDA5 with a gain of function mutation G495R (49,50). Eighty-four percent of these Alus are in the 3'untranscribed region (UTR). MDA5 G495R is representative of mutations in MDA5 leading to aberrant activation of its signaling activity, resulting in a spectrum of immune disorders, such as systemic lupus erythematosus, or Aicardi-Goutières syndrome (51). *In vitro*, these MDA5 variants display more efficient filament formation on dsRNA and high basal signaling activities in the absence of viral infection due to misrecognition of cellular RNAs, resulting in self-triggered signaling (49). Adenosine-to-inosine modification by the adenosine deaminase acting on dsRNA (ADAR) has been found to block the recognition of dsRNA by MDA5. A defect in ADAR1 results in aberrant activation of MDA5 by cellular dsRNAs formed by Alu retroelements (49,52-54). We recently observed in an experimental animal model of asbestos-induced mesothelioma development (21), that asbestos increased the levels of RNA mutations and the most abundant changes were A to G mutations resulting from ADAR activity (55).

Aberrant activation of MDA5 is also observed due to increased synthesis of ERV (*Figure 2C*) upon suppression of DNA methyltransferases (56,57). Interestingly, YAP induces Dnmt3l (58) which although being inactive, stimulates the activity of the others DNMT and is important for the maintenance of embryonic stem cells. Recently, in our own model of mesothelioma development (21) we observed a significant (P=0.0008227, FDR=0.001586) 6.5-fold increase of *Dnmt3l* expression in mesothelioma tumors, consistent with activation of YAP signaling, when compared to inflamed tissue. In the same experimental model *Dnmt1* and *Dnmt3b* are also significantly upregulated, consistent with the observation of methylation of some tumor suppressor genes at early stages during mesothelioma development as documented by increased levels of DNA methylation at *ink4a* locus after mice exposure to asbestos (59). On the other hand, *Dnmt3a* is significantly downregulated in both, mice (21) and rats (60), exposed to asbestos. Since *Dnmt3a* is the enzyme responsible for retroviral silencing in somatic cells (61), this may mean that retroviral elements may not be efficiently silenced.

Increased synthesis of ERV is also observed upon suppression of the histone methyltransferase SETDB1 (62) and this may occur in the subset of mesothelioma patients with mutated SETDB1 (41,63-65). Increased ERV expression is also observed by deficiency of the histone demethylase KDM1A/LSD1 activity (66).

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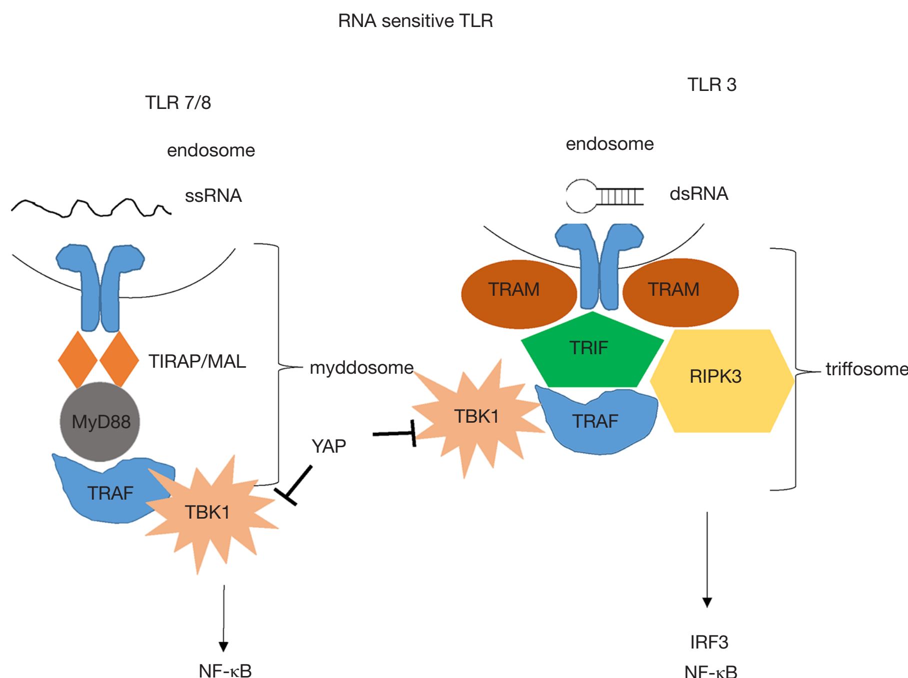

Figure 3 Metabolism and necroptosis regulation downstream endosomal RNA sensors and possible interference of YAP. TLR7/8 induce the assembly of myddosome, upon ssRNA detection. Myddosome assembly occurs around the cytosolic tail of dimerized TLRs present at the plasma membrane or endosomes. The E3 ubiquitin-ligase TRAF6 is present in the myddosome. TRAF6 functions are to stimulate myddosome-associated TBK1 to drive metabolic changes in the cell and to stimulate IKK- and MAPK-dependent transcription factors. TLR3 induces the assembly of triffosome. TIR domain-containing adaptor inducing IFN-β (TRIF) promotes TBK1-dependent gene expression and receptor-interacting serine/threonine-protein kinase 3 (RIPK3)-dependent-necroptosis. YAP inhibits TBK1. YAP, Yes-associated protein 1; TRAF6, TNF receptor-associated factor 6; TBK1, TANK-binding kinase 1; TLR, Toll-like receptor.

The immunostimulatory effect observed by hypomethylating agents such as decitabine and azacytidine is currently exploited in combination immunotherapy treatment in different cancers (67) but not yet in mesothelioma although therapeutic approaches exploiting type-I IFN pathway signaling have already been implemented in the clinic (68) or proposed on the basis of preclinical studies (69,70).

Of particular interest in the field of cancer is that increased expression of ERV is also observed with CDK4/6 inhibitors (71). Activation of interferon signaling is observed upon inhibition of CDK4/6 in mesothelioma cells (72). This is important since CDK4/6 inhibitor abemaciclib is currently tested in p16INK4A negative MPM patients [NCT03654833 (MiST) (73).

Endosomal sensors of dsRNA

Endosomal sensing of nucleic acids is based on TLRs (74). TLR3 is a sensor for double-stranded [ds] RNA) (75), while TLR7 (76) and TLR8 are sensors for (single-stranded [ss] RNA) (77) (*Figure 3*). Recently, in our own model of mesothelioma development, we observed a significant increase of *Tlr3*, *Tlr7* and *Tlr8* upon asbestos exposure (21).

Ligand-binding mediates TLR dimerization, leading to the assembly of signaling complexes activating kinases that drive transcription and glycolysis (78). TLR7/8 induce the assembly of myddosome, upon ssRNA detection. Myddosome assembly occurs around the cytosolic tail of dimerized TLRs present at the plasma membrane or endosomes. The E3 ubiquitin ligase TNF receptor-associated factor 6 (TRAF6) is present in the myddosome. TRAF6 functions are to stimulate myddosome-associated TBK1 to drive metabolic changes in the cell and to stimulate IKK- and MAPK-dependent transcription factors. TLR3 induces the assembly of triffosome that are so called because they contain TIR domain-containing adaptor

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inducing IFN-β (TRIF). TRIF promotes TBK1-dependent gene expression and receptor-interacting serine/threonine-protein kinase 3-dependent necroptosis. Nucleic acids have to be processed to nucleic acids and free nucleosides in endosomes to be able to activate downstream signaling. TLR7 dimerization is most efficient in the presence of ssRNA and free guanosine molecules (79,80). Similarly, TLR8 dimers form most efficiently in the presence of ssRNA and free uridines (77). The process by which dual ligands are produced to maximally dimerize TLRs may occur only in endosomes through the actions of acid-dependent nucleases present in these organelles. Cells lacking the lysosomal RNase T2 are defective for TLR8 signaling (77). Additionally, at least in the case of TLR3, the affinity of the sensor for nucleic acids is strongest at acidic pH (81). Single molecules of dsRNA bind TLR3 dimers (82). TLR3 minimally responds to approximately 40 bp dsRNA by a reporter assay (83,84). Experimental evidence suggests activation of type 1 IFN via TLR3 by antracycline treatment (85), although the species activating the signaling is unknown. It has been reported that addition of dsRNA binding domain agonist poly (I-C) synthetic ligand induces cell death in some TLR3-positive mesothelioma cells and the effects are increased by cisplatin pre-treatment in p53 wild-type cells (69). This is consistent with the knowledge about TLR3 induction by p53 (86) .

Perspective and open questions

Knowledge recently acquired and discussed in this review highlights the role of endogenous ncRNA as ligands in innate immunity in mesothelioma. Mesothelioma is the sixth of 31 cancer types with most prevalent 38- ISGs signature (87) and, in a large fraction of ISGs high tumors, no immune cells, possibly contributing to the phenotype, have been detected, indicating spontaneous IFN production by cancer cells per se. This is consistent with recent studies showing that mesothelioma cells maintain the activation of the type-I IFN signaling pathway (20,88). Oncoprint analysis (www.cBioportal.org) of mesothelioma TCGA data (*Figure 4*) of the different components of the ncRNA regulatory networks involved in innate immunity described in this review shows that patients with an activated type 1 interferon signaling (increased expression of ISG) have a tendency to better overall survival. Alterations in ncRNA regulatory networks are frequently observed and there is a statistically significant co-occurrence between alterations of several components of ncRNA regulatory networks (*Table 1*).

The more detailed mechanisms behind the crosstalk between cancer therapy and stimulation of innate immune system by endogenous ncRNA characterized so far are related to irradiation or inhibition of DNA methyltransferase, which are already explored in clinical trials. Few clinical trials have or are exploring synthetic RNA as direct stimulators (89,90). For example, pre-clinical studies have shown that modified poly-IC, a synthetic mimic of dsRNA, increases tumor infiltration by T cells (91) by stimulating MDA5-mediated production of IFN β in vascular endothelial cells, suggesting that this stimulation may improve T-cell based cancer immunotherapy.

Besides being used for therapeutic intervention, the expression of endogenous ncRNA could be used as in mesothelioma as biomarkers e.g., to stratify patients responding to immunotherapy as it has been shown for ERV in clear cell kidney cancer (92).

On the other side cancer cells impairment of type 1 IFN signaling makes them sensitive to oncolytic therapy (93), indicating ways to select the patients.

While several experimental mechanistic data and clinical evidence (94) have highlighted the contribution of endogenous ncRNA for cancer patients to obtain clinical benefits from immunogenic cell death-inducing therapies, several questions still subsist. This includes the extent of the variation of endogenous ncRNA ligands between individuals. Advances in sequencing techniques and global determination of RNA structures in living cells, such as the PARIS technique mentioned in the introduction, will allow the analysis of the functions of RNA structures, therefore laying a foundation for understanding RNA regulatory networks and immune signaling pathways in mesothelioma. However, as previously mentioned ncRNA could already be explored as potential markers for therapeutic intervention such as ERV for the stratification of patients for immunotherapy.

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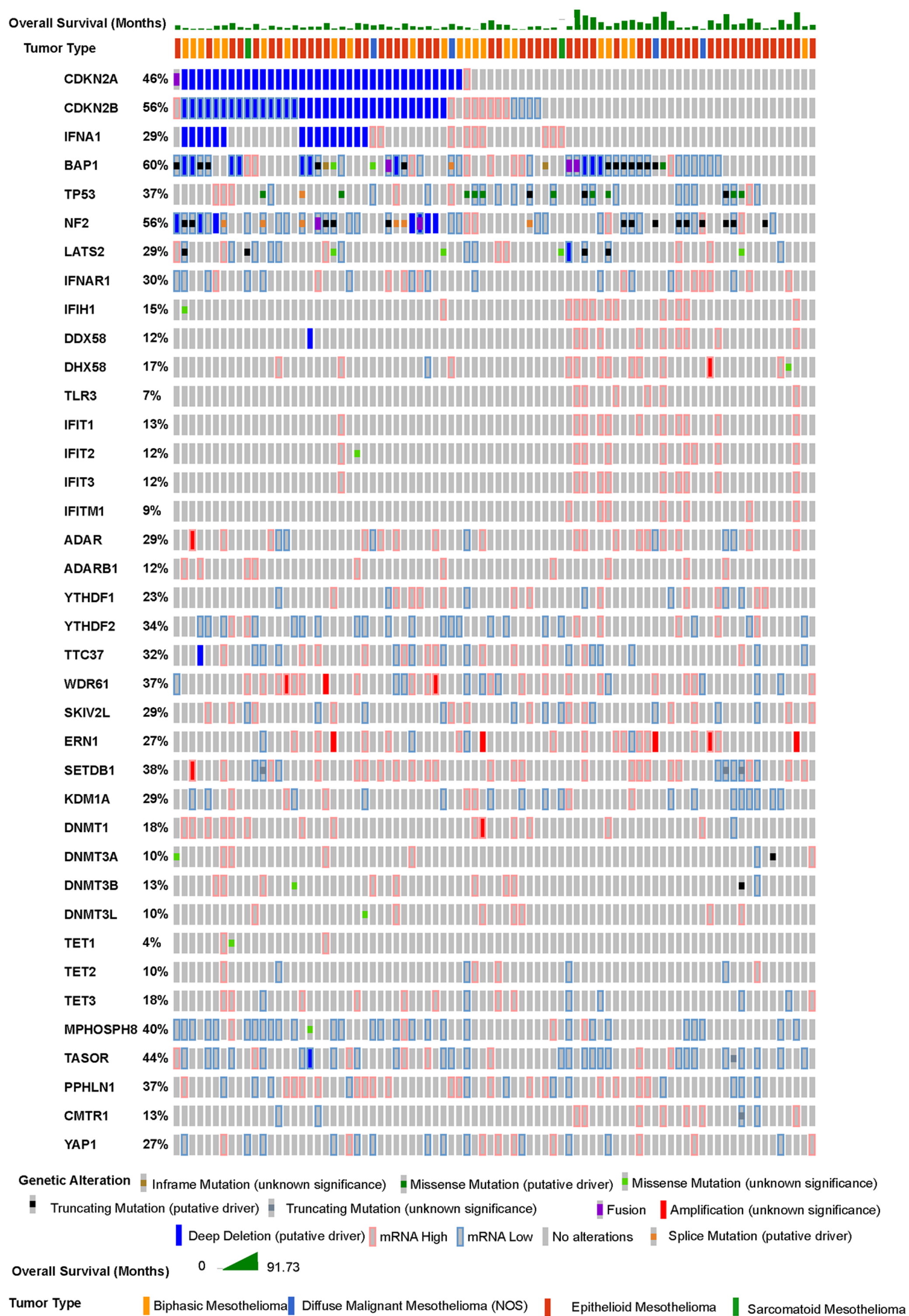

Figure 4 Non-coding RNA regulatory networks and type 1 interferon immune signaling pathways in mesothelioma. "Oncoprint" analysis performed January 26, 2021 using cBioportal (www.cBioportal.org). For m-RNA differential expression we used a z score of 1.2, where the z-score is the standard deviation of static levels of transcript expression in a given case compared to the mean transcript expression in diploid tumors.

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Table 1 Statistically significant co-occurrence alterations in non-coding RNA and innate immune system regulatory networks in mesothelioma (www.cBioportal.org)

A	B	Neither	A Not B	B Not A	Both	Log2 Odds Ratio	p-Value	q-Value	Tendency
CDKN2A	CDKN2B	35	1	9	37	>3	<0.001	<0.001	Co-occurrence
IFIT1	IFIT3	71	1	0	10	>3	<0.001	<0.001	Co-occurrence
DDX58	IFIT1	70	1	2	9	>3	<0.001	<0.001	Co-occurrence
DDX58	IFIT3	70	2	2	8	>3	<0.001	<0.001	Co-occurrence
IFIT2	IFIT3	70	2	2	8	>3	<0.001	<0.001	Co-occurrence
IFIT1	IFIT2	69	3	2	8	>3	<0.001	<0.001	Co-occurrence
IFIH1	IFIT3	67	5	3	7	>3	<0.001	0.002	Co-occurrence
IFIH1	IFIT1	66	5	4	7	>3	<0.001	0.005	Co-occurrence
DDX58	IFIT2	68	4	4	6	>3	<0.001	0.008	Co-occurrence
CDKN2A	IFNA1	39	19	5	19	2.963	<0.001	0.009	Co-occurrence
IFIH1	TLR3	69	7	1	5	>3	<0.001	0.009	Co-occurrence
CDKN2B	IFNA1	33	25	3	21	>3	<0.001	0.009	Co-occurrence
IFIT3	IFITM1	70	5	2	5	>3	<0.001	0.009	Co-occurrence
DDX58	CMTR1	67	4	5	6	>3	<0.001	0.011	Co-occurrence
IFIT1	IFITM1	69	6	2	5	>3	<0.001	0.015	Co-occurrence
TLR3	ADAR	58	0	18	6	>3	<0.001	0.016	Co-occurrence
IFIH1	DDX58	66	6	4	6	>3	<0.001	0.016	Co-occurrence
IFIH1	IFIT2	66	6	4	6	>3	<0.001	0.016	Co-occurrence
IFIT1	CMTR1	66	5	5	6	>3	<0.001	0.017	Co-occurrence
CDKN2A	MPHOSPH8	34	15	10	23	2.382	<0.001	0.017	Co-occurrence
IFIH1	IFITM1	68	7	2	5	>3	<0.001	0.017	Co-occurrence
NF2	IFNAR1	32	25	4	21	2.748	<0.001	0.019	Co-occurrence
DNMT3A	TET1	74	5	0	3	>3	<0.001	0.019	Co-occurrence
CDKN2B	IFIT1	26	45	10	1	<-3	<0.001	0.026	Mutual exclusivity
TP53	DNMT3B	50	21	2	9	>3	0.001	0.040	Co-occurrence
DDX58	TLR3	70	6	2	4	>3	0.002	0.040	Co-occurrence
TLR3	IFIT2	70	2	6	4	>3	0.002	0.040	Co-occurrence
TLR3	IFIT3	70	2	6	4	>3	0.002	0.040	Co-occurrence
CDKN2B	DDX58	27	45	9	1	<-3	0.002	0.049	Mutual exclusivity
CDKN2B	IFIT3	27	45	9	1	<-3	0.002	0.049	Mutual exclusivity
CDKN2B	IFITM1	29	46	7	0	<-3	0.002	0.049	Mutual exclusivity
TTC37	TET3	51	16	5	10	2.672	0.002	0.049	Co-occurrence
TLR3	IFIT1	69	2	7	4	>3	0.002	0.049	Co-occurrence
TLR3	CMTR1	69	2	7	4	>3	0.002	0.049	Co-occurrence
DHX58	IFIT1	63	8	5	6	>3	0.002	0.049	Co-occurrence
CDKN2A	CDKN2B	35	1	9	37	>3	<0.001	<0.001	Co-occurrence
IFIT1	IFIT3	71	1	0	10	>3	<0.001	<0.001	Co-occurrence
DDX58	IFIT1	70	1	2	9	>3	<0.001	<0.001	Co-occurrence
DDX58	IFIT3	70	2	2	8	>3	<0.001	<0.001	Co-occurrence

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