The Role of lncRNAs and miRNAs in Therapy-Induced Senescence in Neuroblastoma

Leila Jahangiri1,2· Tala Ishola3

Accepted: 1 September 2022 / Published online: 17 September 2022
© The Author(s) 2022

Abstract
Purpose of Review Neuroblastoma, a paediatric malignancy of the sympathoadrenal lineage with a variable clinical course, is the most prevalent extra-cranial cancer in children. The majority of multi-modal therapeutics utilised for treating neuroblastoma may drive cells towards cell death or cellular senescence.

Recent Findings Although cellular senescence has been historically regarded as a permanent state of non-proliferation, new evidence supports the notion that this process may indeed be much more dynamic than previously thought. Further, senescent tumour cells may escape treatment and further promote inflammation and migration through their repertoire of secreted molecules, leading to disease relapse.

Summary Given this background, we review here the role of non-coding RNAs inclusive of long non-coding RNAs (lncRNAs) and miRNAs in therapy-induced senescence-related processes in neuroblastoma and discuss how these molecules may be manipulated for therapeutic gain.

Keywords Neuroblastoma · Therapy-induced senescence · lncRNAs · miRNAs

Introduction to lncRNAs and miRNAs in Neuroblastoma and Cellular Senescence

Neuroblastoma (NB) is the most commonly diagnosed extra-cranial paediatric malignancy, disproportionately contributing to circa 15% of cancer-related deaths in children since this cancer only accounts for approximately 8–10% of cancer diagnoses in this age group [1]. The clinical course of this
cancer can vary from relentless progression to spontaneous regression, while high-risk cases undergo multi-modal therapy bearing low success rates and reduced patient survival. Accordingly, high-risk NB patient groups, based on the International neuroblastoma risk group (INRG system), constitute circa half of all diagnosed cases, and a variety of clinical factors including MYCN status, ploidy, segmental chromosomal alterations (e.g. 11q and 3p status) and stage contribute to this stratification among other factors [2]. Staging of NB has also been suggested by the INRG staging system (INRCSS) and includes L1, L2, M and MS groups. Briefly, L1 and L2 describe locoregional involvement in addition to the lack or presence of risk-associated imaging evidence, respectively, while M is largely disseminated and metastatic NB. MS encapsulates metastasis to the bone, liver or skin but not to the cortical bone and is seen in children younger than 18 months [2, 3]. Treatment schemes for NB are based on risk groups and stages. For example, in asymptomatic low-risk groups, surgery and observation may be performed, while for intermediate risk-groups surgery and chemotherapy may be advised. High-risk cases receive many treatment combinations including chemotherapy, surgery, radiotherapy, stem cell therapy and monoclonal antibodies [4, 5].

Further to the molecular and clinical characteristics of NB, cellular senescence is a widely accepted component of human ageing, defined as an irreversible state of non-proliferation and growth arrest which may be a consequence of cellular stress induced by interlinked intrinsic or extrinsic factors such as drug treatment, shortening of telomeres, DNA damage, γ-irradiation, oxidative stress, changes to epigenetic states and the activation of oncogenes [6–8]. As such, cellular senescence can be regarded as a tumour suppressive mechanism due to preventing cell growth and malignant transformation in premalignant to malignant stages. Moreover, the irreversible state of senescence has been challenged by, for example, observations made in therapy-induced senescence (TIS) suggesting that therapeutically induced senescent lymphoma cells could undergo stem-like reprogramming, and upon cessation of treatment, senescent cancer cells could re-enter the cell cycle [6]. Accordingly, in this review, we intend to focus on cells within established tumours which enter a state of senescence following radiotherapy and chemotherapy [7–9]. Based on this, TIS may be initially viewed as a tumour suppressive process to prevent further progression of the tumour [10]. On the other hand, senescence-associated β-galactosidase (SA-β-Gal) has been widely accepted as a marker of senescence, while the secretome of senescent cells may promote invasiveness, inflammation and relapse of tumours [11, 12]. Accordingly, it is thought that persistent inflammatory states increase senescent cells, and the relating immunosuppression can impede the process of clearing these cells by cytotoxic T cells and natural killer (NK) cells, while a subset of senescent cells may recover the ability to self-renew, ultimately defying the purpose of deferring to TIS [6, 13–15]. Collectively, this suggests a strong link between chronic inflammation, senescence and tumour relapse, a topic we will explore in this review.

Apart from the widely investigated and understood factors contributing to TIS and the dynamic role of immunosurveillance in maintaining or suppressing TIS cancer cells, arguably the role of non-coding RNA including long non-coding RNAs (lncRNAs) and miRNAs in TIS may be attractive to the field of NB biology. Notably, lncRNAs are longer than 200 base pairs, while miRNAs are ~18–24 base pairs [16], and both RNA species have been linked to various cancer-related processes in NB [17–20]. For example, NB susceptibility may be linked to the polymorphism of LINCO0673, rs11655237 C>T [21], while invasion and proliferation in NB were influenced by miRNA-34a-5p through the axis of Wnt/β-catenin/ SOX4 [22]. On the grounds of the established dynamics between NB biology and non-coding RNAs, we sought to catalogue and discuss the links between TIS and these non-coding RNAs.

**Senescence in NB, the Role of lncRNAs and miRNAs**

**The Characteristics of Senescent Cancer Cells**

Historically, senescence was defined as a terminal state of cell cycle arrest with reduced and limited proliferation capacity [23]. More recently, senescence has been defined as a state of stress response where cells are metabolically active while having undergone terminal cell cycle arrest. The latter clause has, however, been challenged in B-cell lymphoma [6, 24], since these cells may reprogramme and acquire stemness and self-renewal capacities [6, 24, 25]. The mechanistic underpinning was revealed in Eμ-myc transgenic mouse models for senescent and non-senescent B-cell lymphoma, where TIS cells re-entered the cell cycle, and increased Wnt signalling and stem cell signatures. Senescence-released lymphoma cells displayed greater clonogenic and tumour-initiating potential than their never-senescent counterparts that had also received chemotherapy [6]. Also, Saleh and colleagues revealed that a subset of senescent cancer cells may acquire the ability to self-renew in non-small cell lung cancer, and breast and colon cancers following TIS induced by doxorubicin or etoposide [14, 15]. In our opinion, these studies have challenged the previously held paradigm of the terminal cell cycle exit of senescent cells.

Moreover, senescent cells share common characteristics including growth arrest, alterations to chromatin states and decreased levels of lamin-B1 [26–28]. Molecular factors involved in senescence may be p21, p27 and p16^{INK4a}.
(p16, encoded by INK4a/ARF). In turn, p21, p27 and p16 may inhibit CDK2, CDK2 and CDK4/6, respectively. Reduced CDK4 leads to reduced levels of phosphorylated retinoblastoma (pRb), a protein which through its non-phosphorylated form would usually interact with E2F transcription factors and lead to cell cycle arrest [26–28]. Further, it is proposed that perhaps p53 and p21 induce senescence, while p16 may maintain this state [29], while senescent cells express SA-β-Gal [27]. Accordingly, the repertoire of senescent cell-secreted molecules includes vesicles, non-coding RNA, enzymes, cytokines and growth factors collectively termed the senescence-associated secretory phenotype (SASP) [30]. From a cellular metabolic view, senescence induced by cytotoxic drugs in p53-competent cells can involve Akt/mTOR signalling pathway and lead to changes in the chromatin state [24, 29, 31–33]. For example, H3K9 trimethylation markers, suggestive of repressed chromatin may be established around E2F target genes, leading to a senescent state [34]. We have summarised the generic molecular landscape associated with senescence including TIS (Fig. 1).

In addition to the well-known players of senescence, numerous non-coding RNAs may also affect this process and promote or suppress neuronal senescence. For example, the neuroprotective role of miR34a in SH-SY5Y cells in association with lithium was established using hydrogen peroxide (H2O2) to simulate neural injury and stress-induced premature senescence via the production of reactive oxygen species (ROS) [35••]. Various senescence readouts used in this study included changes to p21, p16INK4a, SA-β-Gal, staining of heterochromatin foci linked to senescence and Sudan Black B, while cellular proliferation was assayed using BrdU [35••]. This study showed that lithium restored both cell proliferation and cell cycle arrest induced by H2O2. The latter was established by observing increased p53, p21 and p16 levels. Further, lithium attenuated oxidative stress induced by H2O2, while also modulating miR34a and Sirtuin1 (SIRT1) that are associated with ageing and longevity. Collectively, lithium suppressed NB senescence, partially mediated by miR34a through the miR34a-SIRT1-p53 axis [35]. Concerning the topic of this review, we have henceforth focused on the tumour suppressor or tumour promoter roles of lncRNAs and miRNAs in NB and investigated the role of SASP and the immune system in impacting NB senescence. The role of various non-coding RNAs and other molecules discussed in this review has been summarised in Table 1.

Fig. 1 The molecular landscape of senescence and therapy-induced senescence intended in this study. NB tumour cells exposed to standard therapy regimens may enter a state of senescence which may trigger an immune response. Senescent tumour cells have been depicted in blue, while immune cells have been shown in yellow. TIS may lead to the increase of the levels of Akt/mTOR, p53, p21, p27 and p16INK4a, while levels of CDK2/4/6 and pRb may be decreased. In addition, senescence-associated β-galactosidase (SA-β-Gal) may be a useful marker of senescence. A senescent tumour cell may exit the cell cycle; however, new evidence suggests that this process is more dynamic than previously anticipated.
| Non-coding RNA or other molecules and processes | Mechanism influencing NB senescence | Molecules assayed | Cancer type | Reference |
|-----------------------------------------------|------------------------------------|-----------------|------------|-----------|
| miR34a                                        | Neuroprotective role of SH-SY5Y cells through the miR34a-Sirt-p53 axis in association with lithium | p53, p21, p16INK4a and SIRT1 | NB | [35••] |
| Long-term radiofrequency electromagnetic fields (RF-EMF) | SH-SY5Y cells showed reduced growth and proliferation | Cell cycle assay, Bax and BCL2 (apoptosis), γH2AX (Ser-139 phosphorylation) Akt/mTOR (e.g., p-mTOR) p53, phosphorylated p53, p21, p27 and pRb | NB | [36] |
| miR34a                                        | miR34a led to cell cycle arrest and a reduction in proliferation | p53/p21 and SIRT1 | Oesophageal squamous cancer | [37] |
| p53-dependent miRNAs (e.g., miR-222, miR-192 and miR-145) | NB differentiation induced by retinoic acid and brain-derived neurotrophic factor | p53 | NB | [19] |
| p53-independent miRNAs (e.g., miR-193a-5p, miR-199a-5p and miR-146a) | The overexpression of miR-885-5p led to the inhibition of growth and survival; growth arrest in p53-competent NB cell lines, while apoptosis was triggered in p53-incompetent cell lines; the down-regulation of CDK2 and MCM5 was observed | Cell cycle assay, p21, p53, CDK2 and MCM5 | NB | [38] |
| DICER                                        | NB differentiating cells defer to senescence when DICER reduced | p53 | NB | [19] |
| miR-885-5p                                     | The overexpression of miR-885-5p led to the inhibition of growth and survival; growth arrest in p53-competent NB cell lines, while apoptosis was triggered in p53-incompetent cell lines; the down-regulation of CDK2 and MCM5 was observed | p53 | NB | [38] |
| miR-885-5p                                     | miR-885-5p activated the p53 transcriptional programme including IGFBP3, PPA2B and PTPRE | | NB | [38] |
| NBAT1                                         | NBAT1 mediated p53 target gene regulation (e.g., p21, MDM2 and GADD45A) and directly regulated subcellular levels of p53 | p53, p21, MDM2 and GADD45A | NB | [39] |
| HNF4A-AS1 and miRNA-409-5p                                   | miRNA-409-5p interacted with HNF4A-AS1 to enhance the translation of sPEP1, while sPEP1 protected against cellular senescence and promoted metastasis | SMAD4, genes involved in cancer progression and stemness | NB | [40••] |
| miR-380-5p                                     | miR-380-5p inhibition can lead to the activation of p53 in NB leading to apoptosis | RAS and p53 | NB | [41] |
| HuD                                           | shRNA-mediated knockdown of HuD led to the expression of senescent-related signatures in mouse Neuro2a cells | SA-β-Gal, p16INK4a, ROS, CCL2, CCL20, CXCL2 and IL-6 | NB | [42] |
| MALAT1 and miR-92a                              | Low-dose chemotherapy induced senescence leading to the increased secretion of a ligand of natural killer group 2D (NKG2D), MICA/B through the MALAT1/miR-92a/ADAM10 axis, and promoted immune evasion | MALAT1, miR-92a and ADAM10 | NB | [43••] |
The Tumour Suppressor Roles of miRNAs and IncRNAs in NB Through Inducing Senescence

As discussed, therapy may trigger senescence as an important step in preventing cancer cell proliferation and malignant progression. Consistently, the effect of long-term radiofrequency electromagnetic fields (RF-EMF) on NB cell lines including SH-SY5Y was investigated, and the results revealed that their growth rate was dramatically reduced following exposure to these waves [36]. RF-EMF treatment, with a dose set at 1760 MHz with 4 W/kg for 4 h per day for 4 days, induced G0/G1 cell cycle delay in SH-SY5Y cells. Interestingly, neither apoptosis nor DNA damage was the underlying mechanism contributing to reduced cellular proliferation, since neither γH2AX (Ser-139 phosphorylation, a marker of DNA double-strand break) nor apoptosis was elevated in these cells (e.g. Bax levels were reduced). Contrastingly, signalling molecules including Akt/mTOR were altered (e.g. phospho-mTOR was elevated) in addition to increased levels of p53 and phosphorylated p53 [36]. Delving deeper into the mechanisms revealed that Akt/mTOR activation triggered p53 and also led to the activation of p21 and p27, decreasing levels of CDK2 and CDK4. Also, reduced pRb at Ser807/811 and a consequent cell proliferation reduction ensued [36] (Fig. 1). In our opinion, this study confirmed the molecular characteristics of senescent NB cells, the molecular players of radiotherapy-induced senescence and the cellular response mechanisms involved [44].

Non-coding RNAs may impact TIS and can lead to the occurrence of stable disease with the potential for relapse rather than tumour regression, impacting therapy success. In evidence, the role of miR34a, a member of the p53 transcriptional network, was investigated in oesophageal squamous cancer representative cell lines including ECa-109 (p53 wild-type) and KYSE-410 and KYSE-450 (p53 mutant) treated with Adriamycin, a chemotherapeutic agent inducing DNA damage [37]. Results showed that miR34a induced by DNA damage was linked to both duration of treatment and p53 expression. For example, ECa-109 cells showed reduced proliferation following Adriamycin treatment, while the p53-mutated cell lines did not show significant changes at similar doses but a trend for inhibition at higher doses of Adriamycin [37]. Further, the expression of miR34a in ECa-109 led to reduced proliferation, and senescence assayed by SA-β-Gal. The underlying mechanism of action of miR34a was revealed as the upregulation of p53/p21 and the downregulation of SIRT1 in the p53-competent ECa-109 cell line. Interestingly, no changes were detected in apoptosis and DNA damage markers such as caspase 3 and Poly ADP-ribose polymerase (PARP), respectively, suggesting the phenotype was senescent specific [37]. In our opinion, this study elegantly linked miRNAs to TIS and elucidated p53-dependent, apoptosis-independent mechanisms of senescence induced by Adriamycin.

Specifically, in NB, differentiation-based treatment may also induce stress in cells, and non-coding RNAs may be indicated in this process. In evidence, the process of NB differentiation induced by 5 days of retinoic acid (RA) exposure, followed by brain-derived neurotrophic factor (BDNF) treatment, led to altered expression of numerous miRNAs in a p53-dependent (e.g. miR-222, miR-192 and miR-145) and p53-independent (e.g. miR-193a-5p, miR-199a-5p and miR-146a) fashion [19]. This distinction was made by exposing differentiating SH-SY5Y cells to either a p53 stabiliser (CP-31398) or a p53 inhibitor (Pifithrin-α), whereby CP-31398 and Pifithrin-α increased and decreased the expression of the p53-dependent miRNAs (e.g. miR-222, miR-192 and miR-145), respectively. This highlighted the significant role that p53 plays in senescence. Further, the knockdown of DICER, a molecule involved in pre-miRNA processing, led to differentiating NB SH-SY5Y cells deferring to cell senescence marked by increased SA-β-Gal activity. In our opinion, this study outlined the miRNAs perturbed during NB differentiation and the role of p53 in this process but also emphasised that miRNA processing mechanisms are tightly linked to senescence, and the loss of cellular ability to process miRNAs may indeed lead to senescence as opposed to differentiation [19] (Fig. 2a).

Apart from the role of miRNAs in TIS in NB, other factors may also trigger non-coding RNAs that are linked to NB senescence. In NB patients with no MYCN amplification, segmental 3p deletion is often observed, leading to poor outcomes in these patients [45]. This suggests that tumour suppressor transcripts may be generated from these loci, and a candidate may be miR-885-5p since it was lower in aggressive tumours as opposed to those bearing favourable prognoses [38]. One study revealed the role of this non-coding RNA in TP53-competent (e.g. KELLY, SH-EP and IMR32) and TP53-incompetent (SK-N-BE (2)) NB cell lines. For example, miR-885-5p expression preferentially led to the inhibition of growth and survival, whereby G0/G1 growth arrest was observed in TP53-competent NB cell lines, while apoptosis was triggered in TP53-incompetent cell lines [38]. Further, miR-885-5p induced p21 and p53 in TP53-competent cells, leading to G0/G1 arrest, while miR-885-5p overexpression led to the downregulation of CDK2 and MCM5 by directly binding to their 3′-untranslated region (3′-UTR) [38]. In support of this, CDK2 and MCM5 knockdown generated the phenotype induced by miR-885-5p activation, where growth arrest was induced in a p53-dependent manner. Moreover, miR-885-5p activated the p53 transcriptional programme including IGFBP3, PAP2B and PTPRE, suggesting that non-coding RNA targets of p53 may implement its senescence-related...
In our opinion, this study very elegantly revealed the tumour suppressor role of miR-885-5p in NB in a p53-dependent manner and could be a useful therapeutic target [38] (Fig. 2b).

In agreement with this study, the role of NBAT1 generated from the 6p22.3 loci, a p53-responsive tumour suppressor IncRNA in NB, was reported whereby; NBAT1 increased sensitivity to genotoxic drug treatment (e.g. doxorubicin), while the loss of this IncRNA led to reduced sensitivity to treatment in NB cell lines including SH-SY5Y. Additionally, reduced NBAT1 expression predicted tumour proliferation and poor patient survival [46]. The mechanism was that NBAT1 mediated p53 target gene regulation (e.g. p21, MDM2 and GADD45A) and also directly regulated subcellular levels of p53 [39]. Reduced NBAT1 expression led to altered p53 levels in the nucleus, mitochondria and cytoplasm, and this was executed in a CRM1-dependent fashion, a protein involved in nuclear transport activities [39]. CRM1 inhibition improved the nuclear functions of p53, while increased p53 stability induced through MDM2 inhibition restored drug sensitivity. As expected, inhibiting both CRM1 and MDM2 further enhanced drug sensitivity. These studies reveal the role of NBAT1 within the axis of NBAT1/p53/CRM1/MDM2 as a promising tumour suppressor in NB potentially impacting TIS [39].

In conclusion, although inducing senescence is widely viewed as a tumour-suppressive mechanism, the caveat is that senescent cells may re-enter the cell cycle, proliferate and lead to relapse. Therefore, inducing senescence as a therapeutic strategy might not be failsafe, and instead, we propose that sensitising senescent cells to treatment may be a better option.
The Tumour-Promoting Roles of miRNAs and IncRNAs in NB

In contrast to the studies mentioned that encouraged NB cell senescence as a means of protection against malignant progression, it is plausible that non-coding RNAs can prevent inducing senescence and instead promote cancer progression and metastasis. In evidence, the role of a small peptide (sPEP1) encoded by an IncRNA, hepatocyte nuclear factor 4 alpha antisense RNA 1 (HNF4A-AS1) in NB, was reported [40••]. miRNA-409-5p was shown to interact with HNF4A-AS1 to enhance the translation of sPEP1, while sPEP1 protected against cellular senescence and promoted metastasis and growth in NB cell lines such as SH-SY5Y [40••]. This was accomplished by sPEP1 binding to translation factors (including eEF1A1), the reduced transactivation of SMAD4 and the increase of the transcriptional output of genes involved in cancer progression and stemness. Further, sPEP1 encouraged physical interactions between SMAD4 and eEF1A1. As expected, sPEP1 knockdown led to reduced NB metastasis and self-renewal, while the overexpression of sPEP1 and eEF1A1 predicted poor prognosis [40••] (Fig. 3a). In our opinion, the oncogenic role of HNF4A-AS1-encoding sPEP1 in NB can be a therapeutic target in this cancer and exploited for therapeutic gain, while bypassing senescence.

Similarly, a study showed that miR-380-5p could suppress p53 by binding to the conserved region in its 3’-UTR, whereby inhibiting this miRNA led to p53 activation and induced apoptosis in NB. The endogenous levels of miR-380-5p could inhibit p53 and apoptosis in mouse embryonic stem cells, while the ectopic expression of this miRNA could suppress p53 [41]. Further, miR-380-5p levels decreased following cellular stress induced by ultraviolet (UV) light, while the overexpression of this miRNA attenuated cell death following UV light or cisplatin treatment. In addition, miR-380-5p could cooperate with other oncogenes such as RAS to inhibit senescence and promote tumour transformation and growth [41], while the expression of miR-380-5p was linked to poor prognosis in MYCN-amplified NB. In our opinion, the role of this miRNA as a tumour-promoter is significant, and may be a therapeutic target for p53 activation [41], while the modulation of sPEP1 and miR-380-5p could be promising therapeutic targets for NB.

In conclusion, the tumour-promoting role of various non-coding RNAs in preventing TIS is therapeutically interesting since this mechanism may provide a loophole for senescence prevention, yet allow therapeutic strategies to more effectively target actively cycling tumour cells.

The Secreted Factors of Senescent NB Cells and the Role of the Immune System

As discussed, senescent cells also produce a repertoire of molecules and components termed SASP which may include enzymes, cytokines (e.g. IL-6 and IL-8), chemokines, proteases, angiogenic proteins, regulators of cellular growth and extracellular matrix-associated factors [31]. SASP may be indicated in processes such as angiogenesis, inflammation and tumourigenesis and, therefore, is significant in senescent cell proliferation and relapse and must be considered in drug targeting strategies [32]. Understanding processes and mechanisms that regulate SASP is therefore valuable. Accordingly, in NB, the loss of an RNA-binding protein with transcriptional regulatory roles, HuD, was shown to regulate SASP. shRNA-mediated knockdown of HuD led to the expression of senescent-related signatures in mouse Neuro2a NB cells, including SA-β-Gal, p16INK4a and the production of ROS. Also, target genes of HuD were identified as CCL2, CCL20, CXCL2 and IL-6, which are SASP factors [42]. RNA immunoprecipitation results showed that HuD bound to the 3’-UTR of CC12 in the murine Neuro2a cell line, while HuD knockdown led to increased CCL2 mRNA levels [42]. Consistently, HuD knockout in mice evoked increased CCL2 protein levels. Finally, HuD knockdown in Neuro2a cells showed a higher intensity of SA-β-Gal and CCL2 expression and enhanced sensitivity to γ-irradiation compared to their control counterparts. In our opinion, this study highlighted the significance of HuD as a regulator of SASP and senescence, which may be modulated to increase the sensitivity of NB cells to treatment and may be relevant to TIS [42] (Fig. 3b).

From a functional viewpoint, SASP mediates many of the non-cell-autonomous effects of senescent cells and may play roles in influencing immune cell recruitment or pathological processes such as inflammation, immune cell evasion, tumour promotion and stemness [32]. As mentioned, SASP should in principle attract NK and cytotoxic T cells to eliminate senescent tumour cells [24, 31]; however, this may not completely clear all cells, and the remaining senescent cells may re-enter the cell cycle following a period of cell cycle arrest, hence contributing to tumour metastasis [47].

In agreement with this, a study revealed the molecular players involved in NK immune recognition and immune evasion in NB. Senescent NB cells increased the secretion of a natural killer group 2D (NKG2D) ligand, MICA/B, and promoted immune evasion [43••]. Accordingly, low doses of Aurora-A inhibitor or doxorubicin were used to simulate a model of NB senescence, whereby senescent cells increased MICA/B production and MICA/B recruited
to exosomes enabled the reduction of NKG2D expression in NK cells, resulting in immune evasion. In addition, MICA/B production by senescent NB cells was linked to ADAM10. Further, the combination of both drugs and an ADAM10 inhibitor reduced MICA/B secretion from NB cells and improved NK killing. Finally, ADAM10 was regulated through a dynamic interplay between MALAT1 that sponged the inhibitory effect of miR-92a-3p [43••] (Fig. 3c). In our opinion, the modulation of immune evasion regulated by the opposing roles of miRNAs and lncRNAs (MALAT1/miR-92a/ADAM10 axis) in senescent cells in NB outlines the involvement of these RNA species in this process and warrants further investigation.

In conclusion, the intricate interplay between SASP and the immune system in either promoting or inhibiting immune evasion is an integral part of TIS processes in NB and should be understood in depth. Also, non-coding RNAs are pivotal players in the signalling and regulation of these processes and may fine-tune the secretion and expression of molecules that impact immune evasion.
Discussion

Cellular senescence can be viewed as a stress response mechanism leading to cell cycle exit, accompanied by phenotypic alterations and the production of a repertoire of bioactive secretomes termed SASP [26–30]. In this study, the role of lncRNAs and miRNAs in NB cellular senescence with a focus on TIS was discussed. We initially reviewed the role of lithium in restoring ROS-induced cell cycle arrest, allowing for a neuroprotective role in SH-SY5Y cells through the miR34a-Sirt-p53 axis [35••]. Interestingly, miR34a has been previously reported as a member of the p53 transcriptional network regulating tumour suppression in various cancers including oesophageal squamous cancer [37, 48]. We also reviewed that RF-EMF in SH-SY5Y cells led to altered Akt/mTOR levels; the activation of p53, p21 and p27 and reduced pRb levels [36]. This study brought into focus how RF-EMF as a treatment modality in various cancers [44] may also induce senescence.

Further, we endeavoured to address the role of lncRNAs and miRNAs associated with TIS and focused on both tumour-suppressing and tumour-promoting roles of non-coding RNAs in senescence in NB. For instance, differentiation-based treatment using RA followed by BDNF treatment led to the altered expression of many miRNAs in a p53-dependent manner (e.g. miR-222, miR-192 and miR-145), while the knockdown of DICER switched differentiating SH-SY5Y cells to senescence [19]. The dependence of miRNA function on p53 status was also observed in other studies, whereby enforcing the expression of miR-885-5p led to growth inhibition in p53-competent NB cell lines [38]; inversely, the miR-885-5p expression has been shown to promote colorectal cancer [49]. Further, the targets of miR-885-5p were revealed as CDK2 and MCM5, while miR-885-5p activated the p53-transcriptional programme, suggesting there are multiple layers of the p53-dependent miRNA-led cell cycle progression and proliferation regulation [38]. Finally, we reviewed NBAT1 that directly regulated subcellular levels of p53 in a manner linked to CRM1, and the combination of CRM1 inhibition and the enhanced p53 stabilisation through MDM2 blockage further enhanced drug sensitivity [39]. Consistently, in osteosarcoma, NBAT1 also showed tumour-suppressor effects [50]. Together, these examples outlined the role of tumour-suppressor non-coding RNA in inducing p53-dependent senescence. From a therapeutic standpoint, we propose that the sensitisation of cells to drug treatment may be a better option than inducing senescence.

Conversely, the role of small peptide (sPEP1) encoded by lncRNA HNF4A-AS1 in NB was shown to inhibit cellular senescence and promote metastasis and growth in NB in multiple studies [40••, 51]. Therefore, the oncogenic role of HNF4A-AS1 encoding sPEP1 might be an attractive therapeutic target in NB. On a similar note, we introduced miR-380-5p which can suppress p53 and its expression attenuated apoptosis following exposure to cellular stress [41]. We, therefore, propose that the affective modulation of these oncogenic non-coding RNAs could improve drug sensitivity and treatment outcomes in NB.

Senescent cells can influence their environment by generating a SASP phenotype and contributing to tumourigenesis and inflammation [32]. SASP expression has been linked to RNA-binding protein HuD, a regulator of MYCN in NB [52], where its downregulation increased the levels of SASPs and additionally sensitised cells to senescence inducers such as γ-irradiation [42]. The study was novel in revealing that HuD is a negative regulator of CCL2, a pro-inflammatory chemokine which is upregulated in cancers [42]. Other studies also revealed the relationship between SASP and immune-related processes and non-coding RNAs. For example, NB’s long non-coding RNA MALAT1 was linked to senescence-induced immune escape, while the oncogenic role of this lncRNA has already been reported in other cancers [53, 54]. Low-dose chemotherapy induced senescence where senescent cells increased the secretion of a ligand of NKG2D, MICA/B through the MALAT1/miR-92a/ADAM10 axis, and promoted immune escape [43••]. In our opinion, modulating the MALAT1/miR-92a/ADAM10 axis to reduce MICA/B secretion could boost immune clearance of NB cells, a strategy that could be viewed as immunotherapy in NB.

In conclusion, we discussed the tumour-suppressor and oncogenic roles of non-coding RNA in NB TIS and the implication of SASP and immunosurveillance. A better understanding of these regulatory links may improve the treatment efficacy and quality of life of NB patients.

Author Contribution  LJ and TI wrote, reviewed and edited this study.

Data, Material and/or Code Availability  Not applicable.

Declarations

Conflict of Interest  The authors declare no competing interests.

Human and Animal Rights and Informed Consent  Not applicable.

Open Access  This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, and indicate if changes otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

Springer
References

Papers of particular interest, published recently, have been highlighted as:

• Of importance

•• Of major importance

1. Maris JM. Recent advances in neuroblastoma. N Engl J Med. 2010;362:2202–11.

2. Cohn SL, Pearson ADJ, London WB, Monclair T, Ambros PF, Brodeur GM, et al. The International Neuroblastoma Risk Group (INRG) classification system: an INRG Task Force report. J Clin Oncol. 2009;27(2):289–97.

3. Monclair T, Brodeur GM, Ambros PF, Brisse HJ, Cecchetto G, Holmes K, et al. The International Neuroblastoma Risk Group (INRG) staging system: an INRG Task Force report. J Clin Oncol. 2009;27(2):298–303.

4. Matthyssen KK, Maris JM, Schlieermann GA, Nakagawa A, Mackall CL, Dillon L, et al. Neuroblastoma. Nat Rev Dis Prim. 2016;2:16070.

5. Tolbert VP, Matthay KK. Neuroblastoma: clinical and biological approach to risk stratification and treatment. Cell Tissue Res. 2018;372(2):195–209.

6. Milanovic M, Fan DNY, Bellenki D, Däbritz JHM, Zhao Z, Yu Y, et al. Senescence-associated reprogramming promotes cancer stemness. Nature. 2018;553(7686):96–100.

7. Duy C, Li M, Teater M, Meydan C, Garrett-Bakelman FE, Lee TC, et al. Chemotherapy induces senescence-like resilient cells capable of initiating AML recurrence. Cancer Discov. 2021;11(6):1542–61.

8. Wyld L, Bellantuono I, Tchtonia T, Morgan J, Turner O, Foss F, et al. Senescence and cancer: a review of clinical implications of senescence and senotherapies. Cancers (Basel). 2020;12(8):2134.

9. Wang B, Kohli J, Demaria M. Senescent cells in cancer therapy: friends or foes? Trends in cancer. 2020;6(10):838–57.

10. Schmitt CA, Fridman JS, Yang M, Lee S, Baranov E, Hoffman RM, et al. A senescence program controlled by p53 and p16INK4a contributes to the outcome of cancer therapy. Cell. 2002;109(3):335–46.

11. Zanotti S, Decaestecker B, Vanhuwaert S, De Wilde B, De Vos WH, Sleeman F. Cellular senescence in neuroblastoma. Br J Cancer. 2022;126:1529–38.

12. Faget DV, Ren Q, Stewart SA. Unmasking senescence: context-dependent effects of SASP in cancer. Nat Rev Cancer. 2019;19(8):439–53.

13. Salmena A. Feed-forward regulation between cellular senescence and immunosuppression promotes the aging process and age-related diseases. Ageing Res Rev. 2021;67:101280.

14. Saleh T, Tyyutynk-Massey L, Murray GF, Aloytbi MR, Kawale AS, Elsayed Z, et al. Tumor cell escape from therapy-induced senescence. Biochem Pharmacol. 2019;162:202–12.

15. Birney E, Stamatoyannopoulos JA, Dutta A, Guigó R, Gingeras TR, Margulies EH, et al. Identification and analysis of functional regulatory regions in 1% of the human genome by the ENCODE pilot project. Nature. 2007;447(7146):799–816.

16. Zhu K, Wang L, Zhang X, Sun H, Chen T, Sun C, et al. LncRNA HCP5 promotes neuroblastoma proliferation by regulating miR-186-5p/MAP3K2 signal axis. J Pediatr Surg. 2021;56(4):778–87.

17. Mondal T, Juvvuna PK, Kirkeby A, Mitra S, Kosalai ST, Traxler L, et al. Sense-antisense lncRNA pair encoded by locus 6p22.3 determines neuroblastoma susceptibility via the USP36-CHD7-SOX9 regulatory axis. Cancer Cell. 2018;33(3):417–434.e7.

18. Jauhari A, Singh T, Pandey A, Singh P, Singh N, Srivastava AK, et al. Differentiation induces dramatic changes in miRNA profile, where loss of dicer diverts differentiating SH-SY5Y cells toward senescence. Mol Neurobiol. 2017;54(7):4986–95.

19. Elling R, Chan J, Fitzgerald KA. Emerging role of long noncoding RNAs as regulators of innate immune cell development and inflammatory gene expression. Eur J Immunol. 2016;46(3):504–12.

20. Li Y, Zhao Z-J, Zhou H, Liu J, Liu Z, Zhang J, et al. Additional data support the role of LINCO0673 rs11655237 C>T in the development of neuroblastoma. Aging (Albany NY). 2019;11(8):2369–77.

21. Wang Y, Guan E, Li D, Sun L. miRNA-34a-5p regulates progression of neuroblastoma via modulating the Wnt/β-catenin signaling pathway by targeting SOX4. Medicine (Baltimore). 2021;100(20):e25827.

22. Haylick L, Moorhead PS. The serial cultivation of human diploid cell strains. Exp Cell Res. 1961;25:585–621.

23. Herranz N, Gil J. Mechanisms and functions of cellular senescence. J Clin Invest. 2018;128(4):1238–46.

24. Cahu J, Bastany S, Sola B. Senescence-associated secretory phenotype favors the emergence of cancer stem-like cells. Cell Death Dis. 2012;3(12):e446.

25. Nelson G, Wordsworth J, Wang C, Jurk D, Lawless C, Martin-Ruiz C, et al. A senescent cell bystander effect: senescence-induced senescence. Aging Cell. 2012;11(2):345–59.

26. Baker DJ, Childs BG, Durik M, Wijers ME, Sieben CJ, Zhong J, et al. Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. Nature. 2016;530(7589):184–9.

27. Zon Li. Intrinsıc and extrinsic control of hematopoietic stem cell renewal. Nature. 2008;453(7193):306–13.

28. te Poele RH, Okorokov AL, Jardine L, Cummings J, Joel SP. DNA damage is able to induce senescence in tumor cells in vitro and in vivo. Cancer Res. 2002;62(6):1876–83.

29. Campisi J. Aging, cellular senescence, and cancer. Annu Rev Physiol. 2013;75(1):685–705.

30. Fattá-Labora JA, O’Loghlen A. Classical and nonclassical intercellular communication in senescence and ageing. Trends Cell Biol. 2020;30(8):628–39.

31. Bridger J, Gil J. Senescence and the SASP: many therapeutic avenues. Genes Dev. 2020;34(23–24):1565–76.

32. White RR, Vijn J. DNA double-strand breaks drive aging? Mol Cell. 2016;63(5):729–38.

33. Braig M, Lee S, Lodenkemper C, Rudolph C, Peters AHFM, Schlegelberger B, et al. Oncogene-induced senescence as an initial barrier in lymphoma development. Nature. 2005;436(7051):660–5.

34. Tufekci KU, Alural B, Tarakcioglu E, San T, Genc S. Lithium inhibits oxidative stress-induced neuronal senescence through miR-34a. Mol Biol Rep. 2021;48(5):4171–80. This paper outlined the significance of lithium in suppressing NB senescence and this effect was at least in part mediated through a non-coding RNA (miR-34a) through the miR34a-SIRT1-p53 axis. This paper was integral to the current study.

35. Kim JH, Jeon S, Choi H-D, Lee J-H, Bae J-S, Kim N, et al. Exposure to long-term evolution radiofrequency electromagnetic fields decreases neuroblastoma cell proliferation via Akt/mTOR-mediated cellular senescence. J Toxicol Environ Health A. 2021;84(20):846–57.

36. Ye Z, Fang J, Dai S, Wang Y, Fu Z, Feng W, et al. MicroRNA-34a induces a senescence-like change via the down-regulation of SIRT1 and up-regulation of p53 protein in human esophageal squamous cancer cells with a wild-type p53 gene background. Cancer Lett. 2016;370(2):216–21.
38. Afanasyeva EA, Mestdagh P, Kumps C, Vandesompele J, Ehemann V, Theissen J, et al. MicroRNA miR-885-5p targets CDK2 and MCM5, activates p53 and inhibits proliferation and survival. Cell Death Differ. 2011;18(6):974–84.

39. Mitra S, Muralidharan SV, Di Marco M, Juvvuna PK, Kosalai ST, Reischl S, et al. Subcellular distribution of p53 by the p53-responsive lncRNA NBAT1 determines chemotherapeutic response in neuroblastoma. Cancer Res. 2021;81(6):1457–71.

40. Song H, Wang J, Wang X, Yuan B, Li D, Hu A, et al. HNF4A-AS1-encoded small peptide promotes self-renewal and aggressiveness of neuroblastoma stem cells via eEF1A1-repressed SMAD4 transactivation. Oncogene. 2022;41(17):2505–19. sPEP1 bound to translation factors, reduced transactivation of SMAD4, and increased the transcriptional output of genes involved in cancer progression and stemness. This paper was integral to the current study.

41. Swarbrick A, Woods SL, Shaw A, Balakrishnan A, Phua Y, Nguyen A, et al. miR-380-5p represses p53 to control cellular survival and is associated with poor outcome in MYCN-amplified neuroblastoma. Nat Med. 2010;16(10):1134–40.

42. Ryu S, Jung M, Kim C, Kang H, Han S, Cha S, et al. Loss of RNA binding protein HuD facilitates the production of the senescence-associated secretory phenotype. Cell Death Dis. 2022;13(4):329.

43. Zhang Y, Hu R, Xi B, Nie D, Xu H, Liu A. Mechanisms of senescence-related NK2G2D ligands release and immune escape induced by chemotherapy in neuroblastoma cells. Front cell Dev Biol. 2022 Mar 2;10:829404. In this study, MALAT1 was shown to be linked to senescence-induced immune escape through the MALAT1/miR-92a/ADAM10 axis. Low-dose chemotherapy induced senescence whereby senescent cells increased the secretion of a ligand of natural killer group 2D (NK2G2D). This paper was integral to the current study.

44. Zimmerman JW, Jimenez H, Pennison MJ, Brezovich I, Morgan D, Mudry A, et al. Targeted treatment of cancer with radiofrequency electromagnetic fields amplitude-modulated at tumor-specific frequencies. Chin J Cancer. 2013;32(11):573–81.

45. Plantaz D, Vandesompele J, Van Roy N, Lastowska M, Bown N, Combaret V, et al. Comparative genomic hybridization (CGH) analysis of stage 4 neuroblastoma reveals high frequency of 11q deletion in tumors lacking MYCN amplification. Int J cancer. 2001;91(5):680–6.

46. Pandey GK, Mitra S, Subhash S, Hertwig F, Kanduri M, Mishra K, et al. The risk-associated long noncoding RNA NBAT1 controls neuroblastoma progression by regulating cell proliferation and neuronal differentiation. Cancer Cell. 2014;26(5):722–37.

47. Saleh T, Bloukh S, Carpenter VJ, Alwohoush E, Bakeer J, Darwish S, et al. Therapy-induced senescence: an ‘old’ friend becomes the enemy. Cancers (Basel). 2020;12(4).

48. He L, He X, Lim LP, de Stanchina E, Xian Z, Liang Y, et al. A microRNA component of the p53 tumour suppressor network. Nature. 2007;447(7148):1130–4.

49. Su M, Qin B, Liu F, Chen Y, Zhang R. miR-885-5p upregulation promotes colorectal cancer cell proliferation and migration by targeting suppressor of cytokine signaling. Oncol Lett. 2018;16(1):65–72.

50. Yang C, Wang G, Yang J, Wang L. Long noncoding RNA NBAT1 negatively modulates growth and metastasis of osteosarcoma cells through suppression of miR-21. Am J Cancer Res. 2017;7(10):2009–19.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.