Review

The Long and the Short of It: NEAT1 and Cancer Cell Metabolism

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Simple Summary: Altered metabolism is a hallmark of most cancers. The way that cancer cells regulate their energy production to fuel constant proliferation has been of interest with the hope that it may be exploited therapeutically. The long noncoding RNA, NEAT1, is often dysregulated in tumours. NEAT1 RNA can be transcribed as two isoforms with different lengths, with each variant responsible for different functions. This review explores how the isoforms contribute to cancer metabolism.

Abstract: The long noncoding RNA NEAT1 is known to be heavily dysregulated in many cancers. A single exon gene produces two isoforms, NEAT1_1 and NEAT1_2, through alternative 3′-end processing. As the longer isoform, NEAT1_2 is an essential scaffold for nuclear paraspeckle formation. It was previously thought that the short NEAT1_1 isoform only exists to keep the NEAT1 locus active for rapid paraspeckle formation. However, a recent glycolysis-enhancing function for NEAT1_1, contributing to cancer cell proliferation and the Warburg effect, has been demonstrated. Previous studies have mainly focused on quantifying total NEAT1 and NEAT1_2 expression levels. However, in light of the NEAT1_1 role in cancer cell metabolism, the contribution from specific NEAT1 isoforms is no longer clear. Here, the roles of NEAT1_1 and NEAT1_2 in metabolism and cancer progression are discussed.

Keywords: NEAT1_1; NEAT1_2; metabolism; paraspeckle; cancer

1. Background

In healthy cells, glucose uptake stimulates cell growth through the activation of intracellular signalling pathways, including glycolysis [1]. In cancer, genetic and epigenetic alterations switch these pathways to a permanently “on” state, promoting continuous growth, which depletes key metabolites. As tumour size increases, oxygen availability decreases, limiting the oxygen-dependent final step of the electron transport chain (ETC). This shift from oxidative phosphorylation (OXPHOS) to aerobic glycolysis is termed the Warburg effect [2–4]. Although there is an increase in both glycolytic enzymes and activity in many cancers, the Warburg effect alone does not explain why cancer cells maintain enhanced glycolysis in normoxic cell culture nor in circulating blood [4].

In general, the functions of long noncoding RNAs (lncRNAs) are not well-understood due to their generally low expression levels and high tissue specificity [5]. An exception to this is nuclear enriched abundant transcript 1 (NEAT1) dysregulation in many diseases [6]. The highly conserved, single exon, intergenic lncRNA is transcribed near the multiple endocrine neoplasia locus on human chromosome 11q13.1 [7]. Altered 3′-end processing results in two transcripts: 3.7 kb NEAT1_1 and 22.7 kb NEAT1_2; the latter is a well-documented and essential architectural scaffold for subnuclear paraspeckles (see review by...
McCluggage and Fox, 2021) (Figure 1) [8,9]. A triple-helix structure, processed by RNase P at the 3′-end of NEAT1_2, stabilises the transcript to protect it from degradation [9,10]. Less studied is the shorter, polyadenylated, paraspeckle-independent NEAT1_1 isoform, whose function, until recently, remained elusive. As previously reported, abnormal NEAT1 expression is correlated with several malignancies, and the distinction between the two isoforms has often been overlooked [11]. Hence, the relative contribution of each isoform to metabolic homeostasis, or pathology, requires attention.

Figure 1. The NEAT1 gene gives rise to two isoforms with identical 5′ sequences. The paraspeckle-independent NEAT1_1 undergoes canonical 3′ polyadenylation whilst the blocking of 3′ polyadenylation via competitive binding of hnRNPK to the CFIm complex yields the paraspeckle-essential NEAT1_2. The recruitment of paraspeckle proteins to the hydrophilic and hydrophobic regions of NEAT1_2 leads to phase-separated paraspeckles.

2. NEAT1_1 Enhances the Warburg Effect by Accelerating Glycolytic Metabolite Flux

Although both isoforms are abundant in the nucleus, NEAT1_1 is also exported to the cytoplasm [12]. In 2019, Adriaens et al. suggested that NEAT1_1 is potentially nonfunctional and serves to only keep the transcriptional locus active, making the switch to NEAT1_2 rapidly available during stress induction [13]. However, a recent study has discovered a novel mechanism of action for NEAT1_1 in the cytoplasm, in both in vitro and in vivo breast cancer (BC) models [12]. The authors demonstrated that the translocation of NEAT1_1
from the nucleus to the cytoplasm, through binding with the nuclear speckle-associated protein pinin (encoded by the PNN gene), occurs in a glucose-dependent manner [12,14] (Figure 2). Interestingly, the depletion of pinin reduces cytoplasmic NEAT1 even after glucose stimulation, indicating the importance of pinin in the nucleocytoplasmic transport of NEAT1 [12]. Importantly, in the cytoplasm, NEAT1 interacts with the glycolytic enzymes phosphoglycerate kinase (PGK1), phosphoglycerate mutase (PGAM1), and alpha enolase (ENO1) to promote glycolysis, enhancing growth, proliferation, invasion, and metastasis (Figure 2) [12,15].

Figure 2. Nucleocytoplasmic transport of NEAT1 enhances the Warburg effect via the binding to glycolytic enzymes. TDP-43, ARS2, and CFIm promote the canonical 3' polyadenylation of NEAT1, which can then bind to pinin for nuclear export to the cytoplasm. Once in the cytoplasm, NEAT1 can bind with the glycolytic enzymes PGK1 (A), PGAM1 (B), and ENO1 (C) to enhance glycolytic flux, and hence the Warburg effect, in transformed cells.

PGK1 is constitutively expressed in all somatic and premeiotic cells and is essential in the glycolysis pathway, but it is implicated in cancer as it encompasses many characteristics of an oncogene [16]. This bilobed enzyme has both nucleotide-binding and catalytic domains and is involved in the conversion of 1,3-bisphosphoglycerate (1,3-BPG) to 3-phosphoglycerate (3-PG) in glycolysis (Figure 2A). It also catalyses the first ATP of anaerobic
glycolysis [16,17]. This substrate-level phosphorylation is of great significance in the continuous production of cellular energy under hypoxic conditions [16]. PGK1 also acts as a protein kinase after translocation to the mitochondria, where it directly phosphorylates pyruvate dehydrogenase kinase isozyme 1 (PDHK1) [17]. Phosphorylated PDHK enhances pyruvate dehydrogenase E1α, which inactivates pyruvate dehydrogenase, preventing the conversion of pyruvate to coenzyme A (CoA), thus suppressing mitochondrial pyruvate metabolism and increasing lactate production [18]. This rate-limiting enzyme plays a role in the promotion of tumourigenesis through the activation of oncocgenic pathways, such as Akt/mTOR, Myc, and β-catenin, and post-translational modifications, such as phosphorylation, acetylation, ubiquitination, and succinylation (as reviewed by Liu et al., 2022) [19]. In vitro research by Gou et al. [20] and Wang et al. [21] have shown that the small-molecule inhibitor of PGK1, NG52, had a dose-dependent effect on the proliferation of ovarian cancer and glioma cells, respectively.

PGAM1 is a highly conserved glycolytic isomerase enzyme involved in the conversion of 3-PG to 2-phosphoglycerate (2-PG), whilst also supporting antioxidative defences by reducing mitochondrial reactive oxygen species (ROS) (Figure 2B) [22,23]. Several studies have linked PGAM1 to cancer progression. Earlier works report PGAM1 knockdown by short hairpin RNA (shRNA) results in an increase in 3-PG and a subsequent decrease in 2-PG, whilst also decreasing glycolysis, pentose phosphate pathway flux, biosynthesis, and cell proliferation in diverse solid and leukaemia cell lines [24]. Investigation of dysregulated PGAM1 levels in human urothelial bladder cancer tissues found a positive correlation with histological-grade tumours, when compared to adjacent normal tissue [25]. Loss of functional tumour-suppressor protein p53, encoded by tumour protein 53 (TP53), is common in cancer, and it has been found to upregulate both NEAT1 and PGAM1 [23]. Similar to many metabolic enzymes, PGAM1 asserts a nonenzymatic function, as the physical interaction with checkpoint kinase 1 (Chk1) increases proliferation, specifically in RAS-driven cancer cells [26].

Alpha enolase (ENO1), encoded by ENO1, is another tumour-related, multifunctional protein, which is responsible for the conversion of 2-PG to phosphoenolpyruvate (PEP) in glycolysis (Figure 2C). An increase in ENO1 expression has been reported in human diseases (i.e., systemic sclerosis, type II diabetes mellitus, lupus, Alzheimer disease) and many cancers, as well as being involved in cell growth and hypoxia tolerance [27–34]. Additionally, silencing ENO1 reduces the rate of glycolysis in cell lines, favouring OXPHOS even when glucose influx remains high [35]. ENO1 expression is correlated with colorectal cancer (CRC) progression, and the newly identified protein translational modification, lysine crotonylation, has been identified at lysine residue 420 in CRC cell lines [33]. Interestingly, although ENO1 is reportedly overexpressed in many human diseases and cancers, in non-small-cell lung cancer (NSCLC), ENO1 is downregulated at the protein level even though its mRNA levels remain elevated, suggesting post-transcriptional regulation [36,37].

Given the cancer-specific roles for each of these enzymes, the role that NEAT1_1 plays, either in glycolysis or in sequestering enzymes from other activities, requires further investigation.

3. NEAT1_2 and Paraspeckle Abundance Increase following Stress

First described in 2002 by Fox et al., paraspeckles are discrete, subnuclear bodies, measuring approximately 360 nm in diameter [38–40]. Paraspeckle formation relies solely on the generation of NEAT1_2 in the nucleus, which sets them apart from cytoplasmic stress granules and P bodies, which require multiple proteins and RNA elements to form [8]. Paraspeckle formation occurs only following the recruitment of proteins, such as non-POU-domain-containing octamer-binding protein (NONO), splicing factor proline and glutamine-rich (SFPQ) and fused in sarcoma (FUS) proteins, among others, to the mid-region of NEAT1_2 transcript. Once localised, the high concentration of molecules aggregates to form a distinct spheroid with spatial organisation [41] (Figure 1). Hydrophilic proteins bind 3’ and 5’ regions of the NEAT1_2 transcript to form the paraspeckle shell, whilst the middle segment of the transcript forms the hydrophobic core [8,10,38,42]. Individ-
ual paraspeckles are spheroidal, but during stress conditions, they can be linked together to generate elongated paraspeckle structures [15]. In HeLa cells, there are ~5–20 paraspeckles per nucleus [43]. However, in 2020, Grosch et al. reported that the size of the nuclei likely influences paraspeckle abundance in human pluripotent stem cells [43]. Regardless of basal paraspeckle abundance, their numbers seem to increase during stress, suggesting that \textit{NEAT1}_2 accumulates.

4. What Is Driving the \textit{NEAT1} Isoform Switch?

Since the formation of paraspeckles is dependent on the transcriptional read-through of \textit{NEAT1}_1 to \textit{NEAT1}_2, understanding the mechanistic control of this isoform switch is crucial. Polyadenylation (polyA) signals terminate the primary \textit{NEAT1} transcript, resulting in canonical processing of the 3′ polyA tail and consequently a short \textit{NEAT1}_1 lncRNA [44]. The long \textit{NEAT1}_2 isoform is generated when heterogeneous nuclear ribonucleoprotein K (hnRNPK) competes with cleavage-and-polyadenylation-specific-factor 6 (CPSF6) for nudix hydrolase 21 (NUDT21) binding, inhibiting the CPSF6–NUDT21 (CFIm) complex from forming, and facilitating the 3′-end polyA (Figure 2) [44,45]. In vitro binding assays have demonstrated that inhibiting the formation of the CFIm complex prevents the polyadenylation of \textit{NEAT1}_1 and increases the nuclear levels of \textit{NEAT1}_2 and, thereby, paraspeckles [44,46]. Additionally, recent work has reported that arsenic resistance protein 2 (ARS2) acts as a chaperone, guiding CFIm to the \textit{NEAT1} transcript to facilitate the polyadenylation of \textit{NEAT1}_1 in osteosarcoma cell lines [47]. Furthermore, the knockdown of ARS2 leads to an increase in, and the preferential stabilisation of, \textit{NEAT1}_2 transcripts [47]. Moreover, RNA binding protein (RBP) transactive response (TAR) DNA binding protein 43 kDa (TDP-43) directly represses the formation of paraspeckles, but it increases \textit{NEAT1}_1 transcription by binding the \textit{NEAT1}_1 GU-rich motifs upstream of the polyA site [48,49]. Although TDP-43 has been thoroughly investigated in amyotrophic lateral sclerosis and has been linked to altered miRNA expression, the understanding of its involvement in a cancer context remains limited [49–55]. In summary, the isoform switch from \textit{NEAT1}_1 to \textit{NEAT1}_2 may involve several factors with context-specific roles; their oncogenic relevance requires further clarification.

5. \textit{NEAT1} and Paraspeckles Alter Metabolism via Mitochondria

Mitochondrial function reaches beyond the established role in energy generation. In addition to ATP production, mitochondria generate macromolecules which alleviate mitochondrial stress [56]. Interestingly, the mitochondrial stressors, FCCP, rotenone, and doxycycline, all increase \textit{NEAT1} levels, in part through ATF2-induced \textit{NEAT1} expression [10]. Mito–nuclear communication is crucial for ensuring cellular homeostasis during mitochondrial stress, and recently it has been hypothesised that \textit{NEAT1} and paraspeckles may play a role in mitochondrial homeostasis [57,58]. Mitochondrial fusion and fission are controlled by dynamin-related GTPases MFN1/MFN2 and DRP1, respectively [58]. \textit{NEAT1}-depletion in HeLa and HEK293 cells resulted in mitochondrial elongation and the enhanced retention of mito-mRNAs encoding functional mitochondrial proteins, such as cytochrome c, subunits of NADH dehydrogenases, and carnitine o-palmitoyl transferase [10]. This was further supported by reduced \textit{DRP1} but stable \textit{MFN1} and \textit{MFN2} expression, increased mitochondrial mRNAs (mito-mRNAs) exported from the nucleus, reduced respiration capacity, ATP generation, extracellular acidification rate (ECAR), and proliferation [10]. On the contrary, \textit{NEAT1} overexpression showed an increase in \textit{DRP1} expression and \textit{DRP1} phosphorylation, corresponding to fragmented mitochondria and increased mitochondrial DNA (mtDNA), and this was phenocopied by deleting the \textit{NEAT1}_1 polyadenylation signal [10].

6. Alternative Processing of lncRNAs in Cancer

Many lncRNAs are implicated in cancer, either through direct or indirect processing, as previously reviewed [59,60]. Similarly to \textit{NEAT1}, the noncoding product of a neighbouring
locus metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is retained in the nucleus and is involved in the nuclear architecture as well as in RNA splicing and gene-regulation [61]. Whilst MALAT1 has been found to be overexpressed in 14% of breast tumour samples, an alternatively spliced variant of MALAT1 (∆sv-MALAT1) showed decreased expression in a subset of tumours and shows potential as an individual prognostic factor for BC [62]. Additionally, similar to NEAT1, MALAT1 contains a tRNA-like cloverleaf structure near the 3′ terminus; however, this structure is cleaved [63] and accumulates as MALAT1-associated small cytoplasmic RNA (mascRNA) in the cytoplasm, where it promotes global translation [64] and hepatocellular cancer cell proliferation [65]. Additionally, the lncRNA ANRIL recruits polycomb proteins to regulate target-gene expression, and the overexpression of truncated isoforms has been reported in bladder cancers [66]. Clearly, the function of many lncRNAs is nuanced and not limited to the full-length transcript. Understanding the activities of truncated forms and processed derivatives of lncRNAs will enlighten our understanding of these complex regulatory molecules.

7. Dysregulation of Both Short and Long NEAT1 in Cancer

NEAT1 (without distinguishing between the long and short isoform) is expressed at similar levels in most healthy tissues, but it is reported to have a relatively low expression in the brain, heart, and whole blood [6]. On the contrary, NEAT1 is upregulated in many solid cancers (reviewed in [6,66–70]), and in most cases, it is associated with aggressive disease and poor outcomes [67–69]. However, only a handful of recent studies have established the relative abundance of the two isoforms, despite many reports suggesting that NEAT1 acts as either a tumour suppressor or oncogene, depending on this isoform ratio (summarised in Table 1) [70–74]. In some haematological malignancies, NEAT1 functions as a tumour suppressor, enhancing the expression of NEAT1_2, and thus paraspeckle formation, which may counteract oncogene-induced stressors in cancer [75,76]. NEAT1 levels are upregulated in multiple myeloma (MM) when compared to healthy controls; however, no correlation with patient prognosis has been found [77]. In chronic lymphocytic leukaemia (CLL), the total NEAT1 expression levels remained similar to those of healthy controls, but the expression of NEAT1_2 was found to be significantly higher [77]. On the other hand, in acute and chronic myeloid leukaemia (AML and CML, respectively) and acute lymphoblastic leukaemia (ALL), total NEAT1 levels decrease in patients’ peripheral blood and bone marrow, and this was found to be an essential mediator of apoptosis induced by imatinib in BCR-ABL-expressing cells [75,77–80].

Table 1. Summary of NEAT1 expression studies, as determined by RT-PCR, in various human cancer subtypes, published between 2014 and 2022.

| Cancer Type            | n/Cell Line/Sample Type | NEAT1 Isoforms Investigated | Major Findings                                                                 | RT-PCR Control Gene | Year  | Ref  |
|------------------------|------------------------|-----------------------------|--------------------------------------------------------------------------------|---------------------|-------|------|
| Multiple myeloma (MM)  | n = 46 MM, n = 14 plasma cell leukaemia (PCL) n = 628 from publicly available datasets: #GSE5900 (44 MM, 12 MGUS, 22 healthy donors) #GSE2658 and #GSE24080 | Total NEAT1 and NEAT1_2 NEAT1_1 and NEAT1_2 with RNAseq | RNAseq allowed estimated isoform abundance was based on unambiguously mapped reads. ↑ NEAT1 in tumour samples when compared to healthy controls 90% of total NEAT1 was NEAT1_1. A negative correlation was found between—4NEAT1 and UPR. Neither total NEAT1 nor NEAT1_2 correlated with overall survival or time-to-next-treatment. NEAT1 was not found to be differentially expressed in diverse cell types, i.e., primary vs. secondary cell leukaemia. | Undisclosed         | 2019  | [81] |
Table 1. Cont.

| Cancer Type                      | n/Cell Line/Sample Type | NEAT1 Isoforms Investigated | Major Findings                                                                                       | RT-PCR Control Gene | Year | Ref  |
|----------------------------------|-------------------------|-----------------------------|----------------------------------------------------------------------------------------------------|---------------------|------|------|
| B-cell acute lymphoblastic leukaemia (ALL) | n = 16 blood samples   | Total NEAT1 and NEAT1_2    | ↑NEAT1_1, inferred by the difference in Ct value between total NEAT1 and NEAT1_2.                  | GAPDH               | 2020 | [77] |
| Acute myeloid leukaemia (AML)    | n = 20 blood samples    |                             | ↑NEAT1_1, inferred by the difference in Ct value between total NEAT1 and NEAT1_2.                  |                     |      |      |
| Chronic lymphocytic leukaemia (CLL) | n = 310 blood samples  |                             | ↑NEAT1_1, inferred by the difference in Ct value between total NEAT1 and NEAT1_2.                  |                     |      |      |
|                                  | n = 72 peripheral blood samples | Total NEAT1               | p53 binds to the NEAT1 promotor in CLL and lymphoma.                                               | Lamin B1             | 2015 | [82] |
| Chronic myeloid leukaemia (CML)  | n = 26 peripheral blood samples | Total NEAT1 and NEAT1_2    | ↓Total NEAT1 and ↓NEAT1_2. Silencing BCR-ABL expression total NEAT1 and NEAT1_2 in CML cell line K562, suggesting NEAT1 may regulate BCR-ABL mediated pathways. c-myc represses NEAT1 transcription by binding to promoter. | ACTB                | 2018 | [78] |
| Acute promyelocytic leukaemia (APL) | n = 31 APL and 12 normal blood samples, NB4, NB4-R2, and U937-PR9 cell lines | Total NEAT1 and NEAT1_2 | ↓Total NEAT1 and NEAT1_2 in APL patient samples when compared to normal granulocytes. NEAT1 expression is repressed by PML-RARα fusion gene. NEAT1 expression is involved in the differentiation of APL cells. | ACTB                | 2014 | [75] |
| Thyroid carcinoma (TC)           | n = 98 Peripheral blood and thyroid tissue samples (malignant n = 52, benign n = 46) | NEAT1_2                    | ↑NEAT1_2 in benign vs. malignant thyroid nodules.                                                   | GAPDH               | 2020 | [83] |
| Cancer Type                                      | n/Cell Line/Sample Type | NEAT1 Isoforms Investigated | Major Findings                                                                                                                                                                                                                                                                                                                                 | RT-PCR Control Gene | Year | Ref |
|------------------------------------------------|-------------------------|----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|------|-----|
| Circulating blood monocytes (CBMs) and tumour-associated macrophages (TAMs) | n = undisclosed TPC-1 cell line Bone marrow-derived macrophages and macrophages | Undefined | †NEAT1 expression in TAMs, compared to CBMs. NEAT1 is a direct target of miR-214 in TC cell lines. Knockdown of NEAT1 impairs malignant progression of thyroid papillary carcinoma and tumour growth in vivo. | GAPDH               | 2017 | [84] |
| Anaplastic thyroid carcinoma (ATC)               | n = 25 matched samples SW1736 and KAT-18 cell lines | Total NEAT1 | †NEAT1 in ATC tissues and cells exposed to hypoxic conditions.                                                                                                                                                                                                                                                                                                                                                       | GAPDH               | 2020 | [85] |
| Papillary thyroid carcinoma (PTC)                | n = 20 matched samples NPA87, TPC-1, KAT-5, and HT-ori3 (control) cell lines | Total NEAT1 | †NEAT1 expression in patient PTC samples when compared to adjacent normal tissues. †NEAT1 expression in PTC cell lines compared to control cells.                                                                                                                                                                                                                                                                                                 | GAPDH               | 2018 | [86] |
| Neuroblastoma                                   | Publicly available datasets (total n = 1062): Versteeg (n = 88), Kocak (n = 476), and SEQC (n = 498) | Total NEAT1 and NEAT1_2 (RT-PCR and RNA-FISH) | NEAT1_1 abundance inferred by subtracting NEAT1_2 levels from total NEAT1 levels. †NEAT1_1:NEAT1_2 in aggressive neuroblastoma. †NEAT1_2 and †paraspeckles in nonaggressive neuroblastoma.                                                                                                                                                                                                                     | RPLP0               | 2021 | [87] |
| Breast cancer (BC)                              | MCF-7, MDA-MB-453, MDA-MB-231, SKBR3, and MCF-10A (control) cell lines | Total NEAT1 | †NEAT1 in all cancer cell lines when compared to control cell line. NEAT1 was negatively correlated with miR-448.                                                                                                                                                                                                                                                                                                                        | GAPDH               | 2018 | [88] |
| Cancer Type | n/Cell Line/Sample Type | NEAT1 Isoforms Investigated | Major Findings | RT-PCR Control Gene | Year | Ref |
|-------------|-------------------------|-----------------------------|----------------|---------------------|------|-----|
|             | n = 1065 post-data filtering of TCGA (n = 526), Oslo2 (n = 378), and METABRIC (n = 1904) cohorts. BT474, BT549, HCC1569, Hs578T, MDA-MB-231, MDA-MB-468, MCF7, SK-BR-3, and T-47D cell lines n = 74 BC biopsies and n = 27 non-malignant biopsies | NEAT1_2 NEAT1_1 with RNAseq NEAT1_2 with RNA-FISH | NEAT1_1 expression level determined from polyA-selected RNAseq data from TCGA cohort. NEAT1_1 expression is highest in ER-positive luminal A and B breast cancer. ↑ NEAT1_2 and paraspeckle abundance correlate with high-grade disease (RNA-FISH). ↑ NEAT1_2 in HER2-enriched and luminal B BC in all three cohorts. NEAT1_2 is not expressed at RNA-FISH-detectable levels in normal breast tissue. | Geometric mean of GAPDH, B2M, and RPLP0 | 2020 | [89] |
|             | MCF-7, MDA-MB-231, and MDA-MB-468 cell lines Gene expression data n = 2000 | NEAT1_1 and NEAT1_2 | NEAT1_1 transcription was analysed by using a polyA primer for cDNA generation before RT-qPCR, using primers targeting total NEAT1. NEAT1_2 transcription was analysed by using random primers for cDNA generation before primers specifically targeting the NEAT1_2 region of the transcript. ↑ NEAT1 associated with poor patient prognosis. | RPL11 | 2015 | [90] |
|             | MDA-MB-231 and MCF-10A (control) cell lines | NEAT1_2 with RNA-FISH | ↑ Paraspeckle formation in MCF-7 cell lines when compared to MCF-10A cells. ↑ NEAT1_2 expression after G-quadruplex (G4)-specific stabilization with small molecules. NEAT1_2 expression could be regulated by a G4s. | GAPDH and ACTB | 2021 | [91] |
| Osteosarcoma (OS) | U2OS cell line | Total NEAT1 and NEAT1_2 (RT-qPCR and RNA-FISH). NEAT1_1 in NEAT1_2 KO cells | NEAT1 isoform-specific KO cell lines were achieved using CRISPR-Cas9 technologies. NEAT1_1 levels were unaltered or increased in some NEAT1_2−/− lines. NEAT1_1 localises to nuclear speckles, independent of paraspeckles. | RPLP0 | 2017 | [92] |
| Cancer Type | n/Cell Line/Sample Type | NEAT1 Isoforms Investigated | Major Findings | RT-PCR Control Gene | Year | Ref |
|-------------|-------------------------|----------------------------|----------------|---------------------|------|-----|
| **Cancers** |                         |                            |                |                     |      |     |
| n = 47 biopsies and adjacent matched tissues HOS, SaOS2, MG63, U2OS, and hFOB1.19 (control) cell lines | Total NEAT1 | ▲NEAT1 expression ▲HIF-1α in MG63 cells, and this NEAT1-mediated HIF-1α expression was reversed by miR-186-5p in HOS cells. ▲NEAT1 in OS tissues and cell lines. ▲NEAT1 associated with advanced clinicopathologic features and poor overall survival. NEAT1 promotes proliferation, invasion, and EMT in cell lines. NEAT1 promoted growth in vivo. miR-186-5p is a downstream target of NEAT1 in osteosarcoma. | GAPDH | 2019 | [93] |
| U2OS cell line | Total NEAT1 and NEAT1_2 | Total NEAT1 levels were slightly higher in CBP80-KD and ARS2-KD cells when compared to control KD cells. NEAT1_2 alone 5-fold in ARS2-KD cells, but not in CBP80- or PHAX-KD cells. ARS2 suppresses the formation of paraspeckles. | GAPDH | 2020 | [47] |
| n = 30 paired tissue samples; CAOV3, ES-2, and IOSE80 (control) cell lines | Total NEAT1 | ▲NEAT1 in patient samples and OC cell lines. NEAT1 knockdown with siRNA increased apoptosis and decreased proliferation, colony formation, migration, invasion, and glycolysis. | GAPDH | 2020 | [94] |
| **Ovarian cancer (OC)** | ovarian carcinoma patient specimens (n = 18 responsive, n = 14 resistant) | Total NEAT1 | ▲NEAT1 in treatment-resistant patients when compared to treatment-responsive patients. NEAT1 knockdown enhanced PTX sensitivity in PTX-resistant OC cells. NEAT1 negatively regulates miR-194 expression. NEAT1 sponges miR-194, leading to upregulation of ZEB1 expression. NEAT1 knockdown improved sensitivity to PTX in OC in vivo. | GAPDH | 2017 | [95] |
| **Prostate cancer (PC)** | LNCaP, DU145, and RWPE-1 (control) cell lines | Total NEAT1 | ▲NEAT1 in PCa cells. NEAT1 negatively regulates hsa-miR-218-5p and has-miR-483-3p when compared to normal prostate epithelial cells. | GAPDH | 2022 | [96] |
Table 1. Cont.

| Cancer Type               | n/Cell Line/Sample Type                                                                 | NEAT1 Isoforms Investigated | Major Findings                                                                                                                                                                                                 | RT-PCR Control Gene | Year | Ref  |
|--------------------------|-----------------------------------------------------------------------------------------|-----------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|------|------|
| Primary prostate and bone metastatic tissues | Explant cultures from primary, patient-derived bone metastatic prostate panel primary prostate and bone metastatic tissues Patient-derived xenograft TCGA datasets | Total NEAT1 Total NEAT1_1 (RNA-FISH) | ▲NEAT1 in prostate cancer when compared to normal tissues (from TCGA datasets). ▲NEAT1_1 predicts poor patient prognosis. NEAT1_1 enhances prostate-patient-derived xenograft growth through the post-transcriptional RNA modification N6-methyladenosine (m6A). m6A level of NEAT1_1 correlated to prostate cancer progression and bone metastasis, and negatively correlated to patient survival. | GAPDH               | 2020 | [97] |
| Hepatocellular carcinoma (HCC) | blood samples (n = 36 HCC, n = 36 controls) | Total NEAT1 | ▲NEAT1 in HCC patient samples when compared to healthy controls. miR-129-5p negatively correlated to NEAT1 levels. | GAPDH               | 2019 | [98] |
|                            | n = 62 matched biopsies MHCC97H, MHCC97L, SMCC7721 and LO2 (control) cell lines | Total NEAT1 | ▲NEAT1 in HCC tissues compared to adjacent tissues. ▲NEAT1 correlated with tumour size and vascular invasion. NEAT1 knockdown inhibits proliferation, colony formation, and cell invasion in HCC. miR-613 is a target of NEAT1 in HCC. | GAPDH               | 2017 | [99] |
|                            | n = 28 biopsies and adjacent tissues HepG2, MHCC97L, MHCC97H, and LO2 (control) cell lines | Total NEAT1 | ▲NEAT1 expression compared to matched tumour samples. Patients with NEAT1 expression had HIF-2α expression, whilst patients with—NEAT1 expression (though still significantly higher than matched samples) had —HIF-2α expression. | GAPDH               | 2018 | [100]|
| Gastric cancer (GC)        | n = 140 samples and n = 20 adjacent tissues NCI-N87, SGC-7901, MKN-45, AGS, and GES-1 (control) cell lines | Total NEAT1 | ▲NEAT1 expression in GC cell lines compared to control cell line. NEAT1 regulates expression of EMT-associated genes in GC cells; ↓ in vimentin and N-cadherin, ↑ in Zo-1 and E-cadherin; suggests KD of NEAT1 may inhibit EMT. | GAPDH               | 2016 | [101] |
| Cancer Type | n/Cell Line/Sample Type | NEAT1 Isoforms Investigated | Major Findings | RT-PCR Control Gene | Year | Ref |
|-------------|-------------------------|-----------------------------|----------------|---------------------|------|-----|
| Lung adenocarcinoma (LUAD) | n = 124 biopsies and adjacent tissues A549, CL1-0, and BEAS-2B (control) cell lines | Total NEAT1 | Overexpression rate of NEAT1 in lung cancer samples was 90.3%. Significant positive correlations found between NEAT1 and Oct4 mRNA expression levels. Oct4 directly binds to NEAT1 promoter. Lung cancer cell lines A549 and CL1-0 transiently overexpressing Oct4 induced NEAT1 promoter activity. | GAPDH | 2017 | [102] |
| | A549, H460, H1650, H1975, H1299, and NHBE (control) cell lines TCGA database: n = 687 | Total NEAT1 | ↑NEAT1 expression in all cell lines and patient samples when compared to normal tissue and control cell lines. Positive correlation between ATF2 and NEAT1 expression in LUAD tissues. | ACTB | 2020 | [103] |
| Non-small-cell lung cancer (NSCLC) | A549, H1299, H460, H1975, and BES-2B (control) cell lines | Total NEAT1 | ↑NEAT1 expression in all carcinoma cell lines when compared to control cell lines. NEAT1 promotes growth, migration, and invasion of A549 and H460 cells. NEAT1 directly targets hsa-miR-98-5p, and its expression was significantly downregulated in NSCLC cell lines when compared to normal lung epithelial cell line. MAPK6 is a direct target of hsa-miR-98-5p in NSCLC cells. | GAPDH | 2019 | [104] |
| CRC | n = 30 blood samples, n = 30 controls; validation in n = 100 patients, n = 100 controls. n = 29 matched tissue samples, n = 19, whole blood and tissue samples. HCT116 and LOVO cell lines | Total NEAT1 and NEAT1_2 | Details as to how NEAT1_1 expression was measured were not disclosed. ↑NEAT1 in whole blood of CRC patients when compared to normal controls. Total NEAT1 and NEAT1_2 expression found to be highly accurate in distinguishing CRC patients from normal controls. KD of NEAT1_1 inhibits proliferation and invasion. KD of NEAT1_2 promoted growth. NEAT1 expression was elevated in neutrophils in CRC patients. ↑NEAT1_2 correlated with better overall survival. | ACTB | 2015 | [71] |
| Cancer Type                  | n/Cell Line/Sample Type | NEAT1 Isoforms Investigated | Major Findings                                                                                       | RT-PCR Control Gene | Year | Ref |
|-----------------------------|-------------------------|-----------------------------|-----------------------------------------------------------------------------------------------------|---------------------|------|-----|
| n = 71 tissue samples and n = 61 normal tissue samples from publicly available dataset, RKO, CACO2, SW1116, LOVO, SW480, SW620, HT29, and HCT116 cell lines BALB/c nude mice | Total NEAT1              | ↑NEAT1 associated with poor prognosis in CRC patients. NEAT1 mediates cell proliferation in vitro and tumorigenicity in vivo. KD of NEAT1 significantly inhibited flattening and spreading abilities in HCT116 and SW1116 cells, whilst overexpressing NEAT1 strongly promoted these abilities in HT29 cells. ↑ E-cadherin and —N-cadherin expressed at both mRNA and protein levels in NEAT1 KD cells. NEAT1 OE recovered proliferation potential of CRC cell lines which were impaired by simultaneous downregulation of DDX5. DDX5 correlated with NEAT1 expression in 71 CRC samples. | GAPDH               | 2018  | [105] |
| n = 12 paired patient samples SW480, HT29, and Caco2 cell lines Nude mice (n = 5–7 per group) | NEAT1_2                  | ↑NEAT1 in CRC tissues is negatively correlated with miR-193a-3p. NEAT1 KD ↑ miR-193a-3p expression and attenuates CRC cells. KRAS acts as a target of miR-193a-3p. | GAPDH               | 2019  | [106] |
| GEO databases GSE20916 and GSE9348 n = 100 and adjacent tissue samples SW620, SW480, HCT116, HT29, CaCo-2, LOVO, and Colo205 cell lines | Total NEAT1              | ↑NEAT1 in tumour tissue, when compared to normal tissue, in both independent datasets and in the matched tissue samples. NEAT1 expression correlated with carcinoembryonic antigen (CEA) levels, tumour size, and distant metastasis. ↑ NEAT1 predicts overall survival in CRC patients. NEAT1 regulates cell proliferation and invasion through miR-34a. | GAPDH               | 2019  | [107] |
| Nasopharyngeal carcinoma (NPC) n = 96 NPC and n = 32 nasopharyngeal epithelium tissues | Total NEAT1              | ↑NEAT1 expression in patient samples when compared to normal tissues. NEAT1 expression was negatively correlated with overall survival of NPC patients. | ACTB                | 2019  | [108] |
| Laryngeal cancer (LC) n = 50 paired patient samples TU686, TU177, AMC-HN-8, and 16HBE (control) cell lines | Total NEAT1              | miR-340-5p OE ↑NEAT1 stability via direct binding and consequently ↓NEAT1 expression in LC cells. NEAT1 OE reversed repression of miR-340-5p OE on LC cell proliferation and invasion. | GAPDH               | 2022  | [109] |
Tumour suppressor p53 is regarded as the guardian of the genome, but it is mutated in over 50% of malignancies, enabling cells to escape apoptotic signalling, bypass cell-cycle arrest, and inhibit senescence [112,113]. It is well-established that NEAT1 is induced by p53 binding to the NEAT1 promotor to activate expression [76,112,114]. Interestingly, NEAT1 also promotes p53 and Chk1 through ATR signalling in response to replication stress [76]. Furthermore, in CRC cells, the induction of both NEAT1 isoforms were p53-dependent when exposed to a chemotherapeutic agent and the topoisomerase 2 inhibitor, doxorubicin [112]. In CML, p53 mutations are uncommon [78]; instead, MYC binds to the NEAT1 promotor to enhance expression [78]. Accordingly, Ronchetti et al. reported an increase in total NEAT1 and NEAT1_2 expression in CML patients when compared to normal B-cells [77]. A positive correlation was identified for increased NEAT1 expression and higher histological grades of BC, and NEAT1 levels were elevated in patient plasma and peripheral blood although no relative abundance of NEAT1 isoforms was reported [11]. The same study reported higher expression of NEAT1 in estrogen receptor positive (ER+) breast cancers, when compared to estrogen-receptor-negative- (ER-) BC [11]. Similarly, correlation to lymph node positivity was seen with ER+, but not with ER- [11]. Evidence suggests that NEAT1 point mutations are drivers for breast and prostate cancers, regardless of little change in NEAT1 transcription levels [115–117]. However, a more recent study suggested these mutations were likely caused by transcription errors instead of cancer-specific selection pressures [118]. Regardless, NEAT1 downregulation has been reported in invasive breast carcinoma, oesophageal cancer, pheochromocytoma, and paraganglioma, suggesting NEAT1 plays a tumour-suppressor role in these malignancies [6]. In summary, it is apparent that NEAT1 expression varies greatly between malignancies and that relative isoform abundance may play a key role in disease progression.

8. Considerations for Isoform Detection of NEAT1

Most recent NEAT1 studies have concentrated on the paraspeckle-associated long isoform NEAT1_2, at the risk of overlooking essential roles for the shorter NEAT1_1, paraspeckle-independent isoform. In addition, an analysis of the literature (Table 1) shows the reliance placed upon glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcripts levels for normalizing NEAT1 expression in RT-PCR studies. As new light has been shed on the involvement of NEAT1_1 in glycolysis, a glycolytic housekeeping transcript, such as GAPDH, may not be ideal for normalizing NEAT1 expression in studies related to metabolism.

| Cancer Type | n/Cell Line/Sample Type | Major Findings | RT-PCR Control Gene | Year | Ref |
|-------------|-------------------------|----------------|---------------------|------|-----|
| Laryngeal squamous cell cancer (LSCC) | n = 52 paired tissue samples Hep-2 cell line | ↑NEAT1 expression in LSCC tumour tissues compared to nonneoplastic tissues. NEAT1 expression correlated with T grade, neck nodal metastasis, and clinical stages of LSCC. NEAT1 knockdown inhibited the growth of LSCC xenografts in mice. NEAT1 knockdown induced apoptosis in LSCC cells in vivo. | ACTB | 2016 | [110] |
| Oesophageal squamous cell carcinoma (OSCC) | EC109, EC9706, and HET-1A (control) cell lines | ↑NEAT1 expression in EC109 and EC9706 cell lines. NEAT1 functions as an endogenous sponge for miR-129. | GAPDH | 2017 | [111] |
Inconsistent polyadenylation and contextual processing can make IncRNAs difficult to quantify using standard RT-PCR protocols. Quantifying the relative abundance of NEAT1 isoforms is not straightforward. Kolenda et al. [119] compared cDNA synthesis protocols for various cancer-associated lncRNAs; however, isoform differentiation was not a primary outcome. Similarly, the RNA purification method used can significantly influence the relative abundance of isoforms, with a heating step liberating NEAT1_2 from paraspeckle complexes and increasing yields [120]. While oligo-dT primed cDNA might be used to specifically amplify NEAT1_1 sequences, the presence of poly-A stretches downstream in the NEAT1_2 sequence necessitates the careful calibration of reverse transcription conditions, which is rarely reported. Validated oligo-dT clamp cDNA protocols would be advantageous.

It may be expected that RNA-seq should enable accurate comparison of isoforms; however, many studies report oligo-dT primed libraries, such that the long isoform is under-represented or ignored, and even total RNA-seq data may be influenced by the aforementioned bias in isoform ratios as a result of RNA isolation methods. Newer, long-read direct RNA sequencing methods promise improved qualitative and quantitative data [121].

Visualisation of NEAT1_2, employing RNA-fluorescence in situ hybridization, is often used to quantify paraspeckle abundance and can also be directed to detect NEAT1_1 [13]. Similarly, dCas13 tagging has recently been used to detect both isoforms of NEAT1 in living cells [122].

9. Conclusions and Future Directions

In the context of cancer, NEAT1 may have either a protective, tumour-suppressive role, or a tumour-promoting oncogenic role, depending upon the type of cancer and, most likely, also upon the specific NEAT1 isoform expressed. Many previous studies have concentrated on the total NEAT1 transcription level, rather than on the isoform ratio; hence, isoform-specific contributions are unclear. Improving detection of specific NEAT1 isoforms is crucial in understanding the tumour-promoting vs. the tumour-protective roles of NEAT1 in cancer. Future directions will compare the relevant contributions of paraspeckle-mediated sequestration and epigenetic regulation against the glycolytic influence of the shorter isoform on tumour progression. Whether one or both isoforms are found to be necessary for specifically maintaining neoplasia, that will impact their relative value as therapeutic targets.

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Abbreviations

| Abbreviation | Description                   |
|--------------|--------------------------------|
| ALL          | Acute lymphoblastic leukaemia  |
| AML          | Acute myeloid leukaemia        |
| APL          | Acute promyelocytic leukaemia  |
| ATC          | Anaplastic thyroid carcinoma   |
| ATR          | Ataxia telangiectasia and Rad3-related |
| BC           | Breast cancer                  |
| CBMs         | Circulating blood monocytes    |
| CC           | Cervical cancer                |
| Abbreviation | Definition |
|--------------|------------|
| CFIm         | CPSF6-NUDT21 complex |
| Chk1         | Checkpoint kinase 1 |
| CLL          | Chronic lymphocytic leukaemia |
| CML          | Chronic myeloid leukaemia |
| CoA          | Co-enzyme A |
| CPSF6        | Cleavage and polyadenylation-specific factor 6 |
| CRC          | Colorectal cancer |
| DRP1         | Dynamin-related protein 1 |
| ECAR         | Extracellular acidification rate |
| ENO1         | Alpha enolase |
| ETC          | Electron transport chain |
| FCCp         | Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone |
| FUS          | Fused in sarcoma |
| GAPDH        | Glyceraldehyde 3-phosphate dehydrogenase |
| GC           | Gastric cancer |
| HCC          | Hepatocellular carcinoma |
| KD           | Knockdown |
| KO           | Knockout |
| LC           | Laryngeal cancer |
| IncRNA       | Long noncoding RNA |
| LSCLC        | Laryngeal squamous cell cancer |
| LUAD         | Lung adenocarcinoma |
| mascRNA      | MALAT1-associated cytoplasmic RNA |
| MFN1/2       | Mitofusion protein |
| MM           | Multiple myeloma |
| mtDNA        | Mitochondrial DNA |
| NADH         | Nicotinamide adenine dinucleotide (NAD) + hydrogen |
| NEAT1        | Nuclear enriched abundant transcript 1 |
| NONO         | Non-POU-domain-containing octamer-binding protein |
| NPC          | Nasopharyngeal carcinoma |
| NSCLC        | Non-small-cell lung cancer |
| NUDT21       | Nudix hydrolase 21 |
| OC           | Ovarian cancer |
| OE           | Over-expression |
| OSCC         | Oesophageal squamous cell carcinoma |
| OXPHOS       | Oxidative phosphorylation |
| p53          | Tumour suppressor protein 53 |
| PC           | Prostate cancer |
| PDHK1        | Pyruvate dehydrogenase kinase isozyme 1 |
| PGAM1        | Phosphoglycerate mutase 1 |
| PGK1         | Phosphoglycerate kinase 1 |
| PTC          | Papillary thyroid carcinoma |
| RAS          | Rat sarcoma virus oncogene |
| RBP          | RNA binding protein |
| ROS          | Reactive oxygen species |
| SFPQ         | Splicing factor proline and glutamine-rich |
| shRNA        | Short hairpin RNA |
| TAMs         | Tumour associated macrophages |
| TAR          | Transactive response |
| TC           | Thyroid carcinoma |
| TDP-43       | TAR DNA binding protein 43 kDa |
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