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Insights on ZEB1-AS1: emerging roles from cancer to neurodegeneration

Stephana Carelli[*,#], Federica Rey[#], Erika Maghraby[#], Cristina Cereda[#]

Implications for lncRNAs in the central nervous system: Transcriptional dysregulation is a key contributor to the pathogenesis of a wide range of diseases and long non-coding RNAs (lncRNAs) are highly expressed in the nervous system. Indeed, amongst the over 50,000 lncRNAs expressed in the human genome, more than 40% are specifically expressed in the brain where their roles in brain development, neuron functions, maintenance, and differentiation, are becoming increasingly evident (Zhou et al., 2021). In addition, some lncRNAs were found to have a role in neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), Parkinson's disease, and Alzheimer's disease, along with an interesting link to the physiological aging process (Rey et al., 2021; Zhou et al., 2021). Interestingly, RNA-sequencing analysis conducted in peripheral blood mononuclear cells of sporadic ALS patients and matched controls highlighted 293 differentially expressed lncRNAs contributing to corroborate the importance of lncRNAs in ALS (Gagliardi et al., 2018). Amongst the top ten deregulated lncRNAs identified the most dysregulated antisense of transcription-related genes was Zinc finger E-box binding homeobox 1 antisense 1 (ZEB1-AS1), antisense to the ZEB1 gene (Gagliardi et al., 2018, Garofalo et al., 2020).

ZEB1-AS1: what do we know so far? ZEB1-AS1 is a well-characterized oncogenic regulator over-expressed in different types of cancer such as hepatocellular carcinoma, esophageal squamous cell carcinoma, glioma, osteosarcoma, bladder cancer, colorectal cancer, prostate cancer and B-lymphoblastic leukemia as a promoter of tumorigenesis and tumor progression (Li et al., 2018). ZEB1-AS1 is located on chromosome 10p11.22 and is transcribed from a shared bi-directional promoter with ZEB1 located in the sense strand with respect to ZEB1-AS1 (Li et al., 2018). In cancer cells, the hypomethylation of the ZEB1-AS1 promoter leads to its over-expression. In this condition, ZEB1-AS1 competitively binds to miR-200c which recruits the H3K4 methyltransferase p300 to the ZEB1 promoter, inducing H3K4me3 modification which changes the chromatin status from an inactive state to an active state facilitating ZEB1 transcription (Li et al., 2018). This leads to the upregulation of BMI1, an oncogene member of the polycomb repressive complex 1 family, and a transcriptional repressor (Li et al., 2018). Overexpression of BMI1 has been identified in various human cancers, where it has been correlated to the decrease of glycogen synthase kinase 3β and β-catenin (Li et al., 2018). Indeed, it has been demonstrated that over activation of β-catenin promotes the maturation of colonic crypt stem cells, resulting in a colorectal cancer phenotype by upregulating the expression of target genes such as c-MYC and cyclin D (Zhang and Wang, 2020). For this reason, the ZEB1-AS1/ZEB1/miR200c/BMI1 pathway could exert a guidance role in tumor development.

ZEB1-AS1 in brain cancer: Since cancer and neurodegeneration frequently show different outcomes, i.e., the former acquires mechanisms to resist and evade cell death, whilst the latter is characterized by progressive cellular demise and degeneration. Brain cancer and neurodegeneration appear as non-related disorders (Sharma et al., 2023). Nevertheless, it has been demonstrated that cancer and neurodegenerative disorders also share some common features with inverse behaviors, as if they are two faces of the same medal (Sharma et al., 2023). Interestingly, a percentage of cancer survivors presents a lower risk of developing neurodegenerative disorders, and the potential involvement of specific genes and signaling pathways behind this inverse comorbidity has been recently proposed (Sharma et al., 2023). Recently, an innovative approach was proposed concerning the study of the genomic architecture of patients affected by the two classes of diseases. Indeed, defining the genotype of sporadic and familial cases across the two populations (neurodegeneration and cancer) may improve insight into the shared genetic architecture connecting the disease to a specific phenotype (Sharma et al., 2023).

As a consequence, when thinking of the role of ZEB1-AS1 in neurological disease, the first thing that one can approach is the study of this molecule in brain cancer. Currently, two studies described ZEB1-AS1 role in glioma (Lv et al., 2016; Wei et al., 2018). The study conducted by Lv et al. (2016) highlighted that ZEB1-AS1 is highly expressed in glioma tissues, with a direct correlation with the clinical stage of the tumor and with a negative prognosis. *In vitro* functional studies highlighted that ZEB1-AS1 silencing in glioma cells can inhibit cell proliferation, migration, and invasion, as well as promote apoptosis (Lv et al., 2016). Moreover, the lncRNA was able to regulate epithelial-to-mesenchymal transition through the up-regulation of factors such as ZEB1, MMP2, MMP9, and N-cadherin (Lv et al., 2016). Moreover, the study by Wei and co-authors validated these results and also highlighted that ZEB1-AS1 can act as a competitive endogenous RNA sponge to miR-577 (Wei et al., 2018).

ZEB1-AS1 in neurodegenerative diseases: Very limited evidence is currently present concerning ZEB1-AS1 role in neurodegenerative diseases, but we are strongly convinced that its mechanism of action could be crucial in the regulation of the pathogenesis of neurodegeneration. Indeed, our research group aimed to characterize its mechanism of action in ALS pathogenesis, where ZEB1-AS1 was found to be downregulated (Garofalo et al., 2020).

ZEB1-AS1 downregulation in ALS underlies the importance of the above-cited "inverse association" between cancer and neurodegenerative diseases. For this reason, we started our work with the study of the ZEB1-AS1/ZEB1/miR200c/BMI1 canonical pathway in cancer, and we demonstrated not only that this pathway is dysregulated in peripheral blood mononuclear cells and neural stem cells from sporadic ALS patients, but also that it presents an opposite trend respect to the one observed in cancer (Rey et al., 2023). We further validated these results *in vitro* on the cell line SH-SY5Y where we decreased ZEB1-AS1 expression via RNA interference (Rey et al., 2023). Moreover, we found an increase for ZEB1-AS1 during neural differentiation with an overexpression of β-catenin, highlighting also its aggregation and possible impact on neurite length. Lastly, we demonstrated the role of miR-139 in the modulation of β-catenin (Rey et al., 2023), as this miRNA presents a reduced expression during neural differentiation. miR-139 was found to affect the activation of canonical WNT signaling mediator β-catenin which is a major driver of motor neuron degeneration (**Figure 1**; Hawkings et al., 2022). Interestingly, two papers described a circuitry involving ZEB1, a miRNA, and the well renown ALS-related protein FUS (Dini Modigliani et al., 2014; Zhang et al., 2018). In their work, Dini Modigliani et al. (2014) demonstrated that FUS and two miRNAs (miR-141 and miR-200a) are linked by a feed-forward regulatory loop. Moreover, ZEB1 is a target of miR-141/200a and can act as a transcriptional repressor of these two miRNAs, reinforcing the circuitry (Dini Modigliani et al., 2014). The work by Zhang et al. (2018) also reported the FUS mechanism of action through the binding of miRNA and mRNA targets, reporting as example miR-200c and ZEB1 as a target. Moreover, they show that a truncated mutant form of FUS that leads its carriers to an aggressive form of ALS, R495X, impairs this miRNA-mediated gene silencing (Zhang et al., 2018). These shreds of evidence point to a possible role for the ZEB1-AS1/miR-200c/FUS circuitry in ALS, supporting, even more, the action of ZEB1-AS1 in the pathogenesis of the disease.

Innovative methodologies for ZEB1-AS1 study in neurological disorders: What emerges from the pieces of evidence so far reported is that there is still a lot to be known on the role of ZEB1-AS1 in the central nervous system. Indeed, the functional characterization of lncRNAs remains a major challenge (Wang et al., 2019). One of the most used approaches for the study of lncRNAs relies on clustered regularly interspaced short palindromic repeats (CRISPR)/Cas technology. Indeed, CRISPR/Cas9 can be used to generate lncRNA knockout cell lines and evaluate their function in different cellular processes, whereas CRISPR interference (CRISPRi) and activation (CRISPRa): CRISPRi and CRISPRa can be used to selectively suppress or activate lncRNAs expression, respectively, and study their functions in different cellular processes. Moreover, a crucial point concerns the identification of lncRNA targets, and for this reason, RNA pull-down assays can be used to identify lncRNA-binding proteins and RNAs, which can provide insights into their functions. A similar approach is called chromatin isolation by RNA purification, a technique that uses biotinylated oligonucleotides to capture lncRNAs from crosslinked chromatin and that can be used to identify the genomic regions where lncRNAs interact. Lastly, RNA-FISH (fluorescence *in situ* hybridization) can be used to visualize lncRNA localization in cells and tissues and study their spatial distribution. These approaches can be applied to shed light on ZEB1-AS1 roles in cellular processes (**Figure 2**), through a study of its expression modulation (CRISPR-based approaches), its localization (fluorescence in situ hybridization), or its targets (pull down studies) (**Figure 2**; Wang et al., 2019). Moreover, investigating data repositories containing RNA-sequencing data performed in other neurological disorders could allow the in-depth analysis of ZEB1-AS1 expression in these disorders.

Conclusions: ZEB1-AS1, a well-characterized oncogenic regulator, could also play a crucial role in the regulation of the pathogenesis of neurodegenerative disorders. Indeed, ZEB1-AS1 has also been found to be involved in glioma, but we recently found a role also for the lncRNA in ALS. Indeed, its overexpression appears to lead to a tumorigenic phenotype, whereas its decrease appears to lead to neurodegeneration. Further research is necessary to fully understand the mechanisms of action of this lncRNA in the central nervous system, and single cell data pertaining to ZEB1-AS1 distribution in the brain tissue could be of crucial value. Moreover, it would be definitely interesting to assess the role of ZEB1-AS1 in pediatric neurological diseases and other central nervous system-related disorders where the molecule is yet to be characterized.

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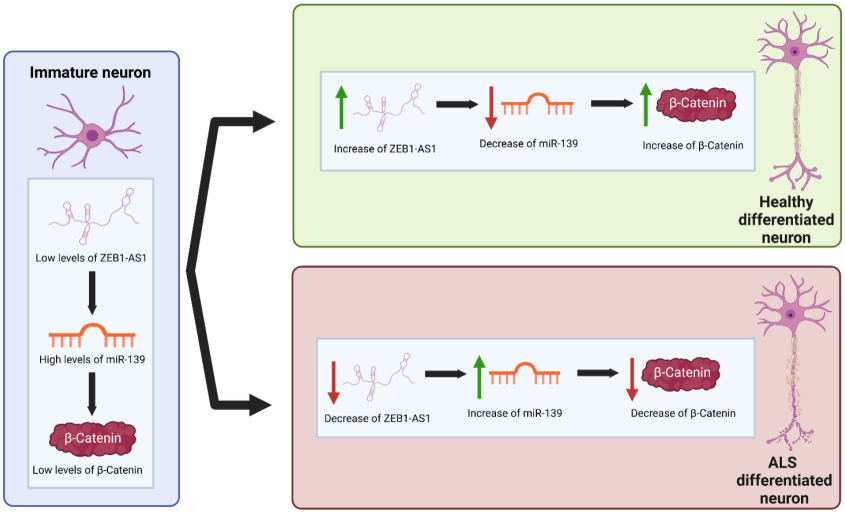

Figure 1 | **Different implications for ZEB1-AS1 in immature and mature neurons.**
The left panel describes the novel ZEB1-AS1 pathway in immature neurons where low levels of ZEB1-AS1 are expressed. Consequently, high levels of miR-139 and low levels of β-catenin are expressed. During differentiation, ZEB1-AS1 expression increases in healthy neurons resulting in downregulation of miR-139 and upregulation of β-catenin (green panel). On the other hand, in ALS differentiated neurons ZEB1-AS1 is downregulated leading to the increase of miR-139 and the decrease of β-catenin (red panel). Created with BioRender.com. ALS: Amyotrophic lateral sclerosis; miR-139: micro RNA 139; ZEB1-AS1: zinc finger E-box binding homeobox 1 antisense 1.

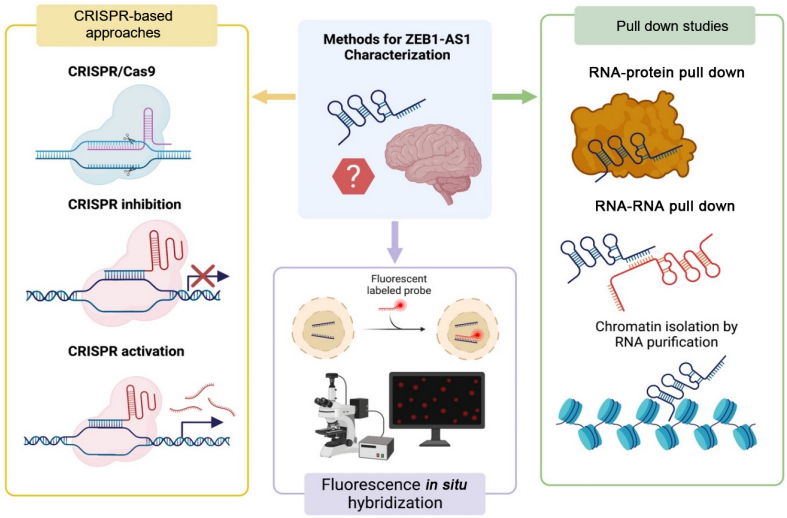

Figure 2 | **Examples of innovative methods for lncRNAs characterization.**
The central blue panel above indicates the possibility of multiple techniques to characterize lncRNAs such as ZEB1-AS1, whilst the violet panel below describes the application of *in situ* hybridization to determine lncRNA localization. The left yellow panel reports the CRISPR-based approaches to modulate the expression of ZEB1-AS1 whilst the right green panel includes the methods to characterize the interactors of ZEB1-AS1. Created with BioRender.com. CRISPR/Cas9: Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas technology; ZEB1-AS1: zinc finger E-box binding homeobox 1 antisense 1.

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