Identification of epigenetic regulators of RNA editing activity

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Rinaldi and Wood Research Groups

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Acknowledgements

I would like to thank Dr Carlo Rinaldi for his encouraging and motivational supervision throughout the project. I would also like to recognise the Wood Research Group, of whom all members in their entirety were always ready and willing to answer questions and provide practical support. Finally, I would like to thank my parents, and my partner for Daniel, for their continued support – both emotional and financial – throughout my studies, and for always reminding me how capable I am.

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1.0 Introduction

1.1 Defining RNA editing

RNA editing is a highly prevalent post-transcriptional modification of mRNAs, tRNAs and rRNAs across all eukaryotes and is pivotal for survival (Ohman and Bass, 2001). Changes in RNA editing have been associated with both the onset of, and contribution to, a diverse range of disorders including cancer, autoimmune, neurological and neurodevelopmental disorders (Christophi and Zaravinos, 2019). This biologically fundamental process can be broadly distinguished into two categories:

1. Insertion/deletion RNA editing – this mechanism of editing changes the length of the target RNA through either deleting or inserting a nucleotide. This leads to a 'frame-shift' altering the entirety of the exon or intron (Bass B, 2000; Gott and Emeson, 2000).

2. Base modification – this mechanism results in a nucleotide substitution, which leads to an alteration in sequence and potentially shape whilst the overall size of the RNA transcript remains the same. This produces one of three outcomes: a silent mutation, in which the altered triplet code leads to the same amino acid therefore not changing the transcript sequence; a missense mutation, where the altered triplet code leads to the production of a different amino acid within the transcript; and, a nonsense mutation, in which the modified base leads to a triplet code which reads as a premature stop codon, resulting in the production of a shorter, and potentially non-functional, protein (Bass, 2000; Gott and Emeson, 2000).

1.2 A-to-I editing

Adenosine to inosine (A-to-I) editing is the most common type of RNA editing (Bazak *et al.,* 2014), being catalysed by the adenosine deaminases acting on RNA (ADARs) enzymes. This family of

proteins comprises four isoforms, ADAR1p150, ADAR1p110, ADAR2 and ADAR3, produced by three genes (Melcher et al.,1996a; Melcher et al., 1996b). Both ADAR1 and ADAR2 are expressed ubiquitously, however ADAR1 is responsible for the majority of editing activity (Lonsdale *et al.,* 2013). Previous studies used x-ray crystallography to characterise a highly conserved positively charged cleft with a catalytic zinc ion at the C-terminal, which allows negatively charged dsRNA to bind to ADAR (Macbeth et al., 2005; Kutton and Bass, 2012). Adenosine is deaminated, forming inosine which is read as guanosine, leading to alterations in splicing and translational processes (Levanon et al., 2004). Recognition of RNA occurs through direct interaction of RNA 2'-hydroxyl groups of RNA by the double-strand RNA binding domains (dsRBDs) – interaction with bases also aids in RNA recognition but to a much lesser degree than the direct interaction of hydroxyl groups by dsRBDs (Stefl *et al.,* 2010). The deaminase activity occurs through a base-flipping mechanism, where the target adenosine residue is approached from the minor groove side via a flipping loop in the N-terminal deaminase domain. The change in helical conformation of the dsRNA shifts bases immediately 5' of the editing site, towards the helical axis (Matthews *et al.,* 2016).

Both ADAR1 and ADAR2 target 'AAG' sites (adenosine-adenosine-guanine) preferentially, editing the middle adenosine. Shifting of the bases towards the helical axis causes the target adenosine residue to 'flip-out' via the minor groove, where it then enters the catalytic deaminase domain of *Adar2*. This complex is stabilised by the interaction of the neighbouring glutamate residue with unpaired bases on the unedited strand of the RNA strand (Egginton *et al.,* 2011; Lehmann and Bass, 2000).

RNA editing is fundamental for survival due to the need to adapt, however it can also result in disease. Recently, researchers have identified ways to employ the RNA editing machinery towards specific targets: for example, the deaminase domain of ADAR has been fused to an RNA-binding protein (RBP) to create an ADAR-RBP fusion protein, and using guide RNA (gRNA) specific codons can be edited at the levels of RNA (Wettengel *et al.,* 2017; Monitel-Gonzalez *et al.,* 2013).

The aim of this project is to identify epigenetic small molecules to regulate endogenous RNA editing.

This work may hold promise for treating diseases in which global A-to-I dysregulation contributes to disease pathology, as discussed later on in this thesis.

1.2.1 ADAR1

The variants ADAR1p150 and ADAR1p110 are two isoforms of the gene *ADAR1*, located on chromosome 1q21.3. These isoforms are a result of alternative start codons and promoter usage (Patterson and Samuel, 1995). ADAR1 contains a C-terminal deaminase domain, three double stranded RNA binding domains (dsRNA) and a Z DNA-binding domain. ADARp150 contains both a Zα and a Zβ DNA-binding domain, with the Zα domain harbouring a Nuclear Export Signal (NES) which allows for the movement of ADARp150 into the cytosol. Whilst ADARp110 only has a Zβ DNA-binding domain and therefore is mainly localised within the nucleus **(see figure 1**) (Gallo *et al.,* 2017). Both isoforms are constitutively expressed and cellular stress leads to phosphorylation of ADAR1p150 by the MKK6-p38-MSK MAP kinases. ADAR1p150 is then able to bind to Exportin-5 and localise within the cytosol (Sakurai *et al.,* 2017). In the cytosol ADAR1p150 competitively binds in particular to the 3'UTR Alu of endogenous dsRNA, inhibiting the binding of *Staufen 1* – mediator of the *Staufen1 mediated RNA decay* (SMD) pathway (Kim *et al.,* 2005). This leads to an antiapoptotic effect as proteins responsible for DNA repair are protected from degradation (Sakurai *et al.,* 2017; Song *et al.,* 2021).

Among its other roles, ADAR1 has a critical function in microRNA (miRNA) maturation. Primary microRNAs (pri-miRNA) are initially transcribed in the nucleus and contain a hairpin loop. This dsRNA structure is recognised by microprocessor complexes which export the pri-mRNA to the cytosol.

Following exportation of the pri-mRNA to the cytosol by microprocessor complexes, DICER cleaves the pri-mRNA into miRNA and siRNA. DICER then facilitates the activation and formation of RNA-induced silencing (RISC) (Michlewski and Cacerers, 2019). Interaction between dsRNA binding

domain 2 of *Adar1* and the *DUF283* and *DEAD-box* RNA helicase domains of DICER allow *Adar1* and DICER to work as a heterodimeric complex (Ota *et al.,* 2013). Interaction with *Adar1* enhances DICERs ability to produce mature miRNA and siRNA; the *Adar1-DICER* complex has also been shown to increase the production of *siRNAs* by as much as four-fold than individual action (Shiromoto *et al.,* 2020). *Adar1's* A-to-I editing activity of pri-mRNA can also lead to either inhibition of DICER, as it is unable to bind to the target sequences or, inhibition of the cleavage of the pri-mRNA by the microprocessor complexes (Yang *et al.,* 2006). This prevents silencing of target genes, and also can result in off-target silencing (Song *et al.,* 2021). Song *et al.* (2021) have shown that *Adar1* activity is determined through the complex it forms: as a homodimer it is able to carry out A-to-I editing affecting the silencing ability of siRNAs and miRNAs, whereas as in a heterodimeric complex with DICER it regulates the production of siRNA and miRNAs.

Whilst ADARp110 contribution to overall activity is still relatively unknown, ADARp150 plays a key role in regulating immunity through suppression of the type 1 interferon (IFN1) response (Licht *et al.,* 2019); Song *et al.,*2021; Crow, 2011). IFN1s are cytokines that have an integral role in the immune response, inflammation, immunoregulation and tumour cell recognition pathways (Razaghi *et al.,* 2021). In order to prevent an autoimmune response to endogenous RNA, ADAR1p150 carries out A-to-I editing of 3'UTR of double stranded endogenous RNAs, which prevents the dsRNA from being recognised by IFN1 (Shiromoto *et al.,* 2021). The majority of immune related A-to-I editing occurs in the thymus, allowing T-cells to recognise edited RNA as 'self' (Egginton, 2011). However, alterations in ADAR1 activity can lead to recoding in additional sites outside of the thymus, this can lead to unrecognised antigens leading to the triggering of autoimmune disease (Eisenberg and Levanon, 2018):

In 2011 a group of diseases categorised as 'type 1 interfernopathies' were identified: these are a group of autoinflammatory diseases in which a lack of IFN1 regulation is directly linked to a decrease in ADAR1 editing activity, of which there are currently 40 identified types (Crow, 2011; Rodero and Crow, 2016; d'Angelo *et al.,* 2021). IFN1 dysregulation leads to an up-regulation of IFN-activating mechanisms and a down-regulation of negative regulatory mechanisms (d'Angelo *et al.,* 2021). Many of these pathologies are associated with increased IFN1 in the CSF (cerebrospinal fluid), and continuous IFN-α production in the astrocytes (Lebon *et al.,* 1981; Campbell *et al.,*1999). As such, many of these diseases have a phenotypical overlap and neuropathological features (Crow, 2003; Crow and Stetson, 2021). Down-regulation of ADAR1 has also been associated with other auto-immune diseases, including *systemic lupus erythematous* (SLE). SLE is an autoimmune disease in which autoantigens trigger an autoimmune reaction. Studies have revealed that there is a significantly increased expression of IFN-inducible genes, high levels of A-to-I editing by Adar1 associated with these genes, and *Adar1* expression itself was shown to be increased. This increase in expression and editing could provide an explanation as to the autoimmune nature of SLE (Roth *et al.,* 2018; Song, 2022).

ADAR1 is well known for its role within both oncogenesis and tumour suppression. Editing activity of ADAR1 has been shown to lead to a "gain of function" effect across a range of cancers including: lung, colorectal, and liver, due to the inhibition of tumour suppression genes (Chen *et al.,* 2013; Hu *et al.,* 2017; Qin *et al.,* 2014; Shigeyasu *et al.,* 2018). This often reduces the sensitivity of tumour cells to immune checkpoints during cell growth, and immunotherapy treatments (Ishizuka *et al.,* 2019). One study showed that loss of function of ADAR1 led to a profound increase in the sensitivity of tumour cells to immunotherapy treatment and also to immune check points within the growth cycle of the cell. This was due to an increase in dsRNA ligand sensing by PKR and MDA5 (IFN1 receptors), resulting in growth inhibition and inflammatory pathway activation within the tumour (Ishizuka *et*

al., 2019). Furthermore, hyper-editing of miRNAs by ADAR1 has been associated with leukaemias as well as multiple myelomas (Wang *et al.,* 2017; Wang *et al.,* 1995; Teoh *et al.,* 2018; Song, 2021).

On the other hand, editing of *CCNI* (encoding cyclin I), a cell cycle regulator, by ADARp110, causes *CCNI* to act as a cancer antigen, which allows T-cells to more effectively identify and kill tumour cells (Song, 2021). A-to-I editing of exon 9 within GABAA receptor-α3s (GABRA3) has been associated with reduced tumour migration and invasion capability of breast cancer cells (Ohlson *et al.,* 2017; Ohlson *et al.,* 2007). GABRA3 has been shown to facilitate cell migration to the brain in breast cancer cells, however ADAR1-edited GABRA3 is not observed in metastatic samples (Gumireddy *et al.,* 2016). Cancer specific RNA editing events are reported as much higher than DNA editing events, such as those mediated by APOBEC3B, and in fact RNA editing is significantly increased in cancer cells vs. non-cancerous cells, particularly in miRNA editing sites (Eisenberg and Levanon, 2018). In melanoma cells A-to-I editing of miR-378 (micro RNA) have been associated with a decrease in metastatic ability of melanoma cells, interestingly in lymphocytes ADAR1 overexpression led to two-times down regulation of mir-222 – increasing overall sensitivity towards cancerous cells – whilst knockdown of ADAR1 led to a four-fold increase in miR-222 and a significant reduction in sensitivity of lymphocytes towards cancerous cells (Song, 2021).

These examples highlight the diverse role of ADAR1 within health and disease, particularly within immune regulation and tumour recognition/suppression. Overall, there is a negative correlation between global A-to-I editing and patient survival in cancer (Paz-Yaacov, *et al.,* 2015). The diversity and involvement of ADAR1 across disease – both in prevention of, and cause – show not only the potential ADAR1 regulation offers in the treatment of disease such as autoimmune diseases and cancer, but also how by studying and mapping editing events of ADAR1 we could identify novel editing sites which are directly linked to pathology of disease – improving both treatment and outcomes for patients.

1.2.2 ADAR2

ADAR2 is primarily localised within the nucleus, similarly to ADAR1 it is constitutively expressed, mainly within the central nervous system (CNS) (Desterro *et al.,*2003). ADAR2 comprises a C-terminal deaminase domain and two dsRNA-binding domains around 65-75 amino acids long rather than three as seen in ADAR1 (St Johnson *et al.,* 1992; Gallo *et al.,* 2017). Nuclear magnetic resonance and X-ray crystallography studies revealed the globular structure of ADAR2, in which the positively charged α-1 helix of the dsRBDs I and II interact with the negatively charged phosphodiester backbone of RNA via the minor grooves (Bycroft *et al.,* 1995; Macbeth *et al.,* 2005; Stefl *et al.,* 2010; Gallo *et al.,* 2017).

ADAR2 is expressed at the highest levels within the CNS, and is associated with regulation of synaptic plasticity and neuronal development (Sinigaglia *et al.,* 2018). Within the CNS, ADAR2 edits the A2 subunit of the glutamate ionotropic receptor AMPA type subunit 2 (*GRIA2*). α-amino-3-hydroxy-5-methyl-4isoxazolepropionic acid (AMPA) receptors mediate fast excitatory synaptic transmission in the CNS, A-to-I editing of *GRIA2* leads to calcium impermeability of ion pore channels within AMPA receptors (Evans *et al.,* 2017). A study involving *Adarb1* knockdown mice (homozygous to human *Adar2*) showed that lack of A-to-I Editing led to progressive seizures and death around 21 days (Higuchi *et al.,* 2000). Another study revealed that mutations within *Gria2* which inhibit the formation of the dsRNA structure also led to calcium impermeability of neurons, and eventual neuronal death (Higuchi *et al.,* 1993); Gallo *et al.,* 2017). This is known as glutamate excitotoxic neuronal death, glutamate is the only neurotransmitter known to cause excitotoxic neuronal death (Hideyama *et al.,* 2010). Across neurodegenerative diseases glutamate excitotoxic neuron death is commonly seen, with A-to-I editing by *Adar2* shown to be downregulated in motor neurons of patients suffering with amyotrophic lateral sclerosis (ALS) (King *et al.,* 2016; Aizawa *et al.,* 2016; Hideyama *et al.,* 2012).

Glaucoma is a neurodegenerative disorder of the optic nerve in which fluid build up within the eye leads to pressure on the optic nerve, damaging the neurons; interestingly, increased calcium permeability of AMPA receptors has been observed in these neurons (Wang *et al.,* 2014). Considering that increased calcium permeability is so strongly linked to down-regulation of A-to-I editing it is not surprising that whilst ADAR2 down-regulation does not cause glaucoma, it clearly can affect the prognosis and progression of disease (Hideyama *et al.,* 2010; Wang *et al.,* 2014). Furthermore, neuronally targeted deletion of *ADAR2* led to motor-neuron degeneration in young mice (Hideyema *et al.,* 2010). This evidence provides a strong support for the school of thought that aberrant ADAR2 editing is implicated across many neurodegenerative diseases in which glutamate excitotoxic neuronal death is observed (King *et al.,* 2016; Aizawa *et al.,* 2016; Wang *et al.,* 2014; Eisenberg and Levanon, 2018). It is not just neurodegenerative diseases that aberrant ADAR2 editing has been linked to: increasing amounts of literature is now being linked to psychiatric conditions, including autism, schizophrenia and bipolar associated disorder (Gallo *et al.,* 2017). Interestingly, ADAR2, as well as ADAR1, has also been linked to SLE: 50% of patients with SLE present with CNS pathology, whilst a further 50% also present with neuropsychiatric pathology (Scolding and Joseph, 2002). Considering these are diseases that are associated with inflammation and decreased neuronal plasticity (Scolding and Joseph, 2002; Eisenberg and Levanon, 2018), it is not surprising that aberrant editing is associated with disease pathology.

Whilst there are many different types of post-transcriptional modifications, ADAR2 carries out the majority of editing within the CNS, and increasing neurologically-based diseases are being associated with aberrant editing events (Eisenberg and Levanon, 2018; Gallo *et al.,* 2017). Therefore ADAR2 should definitely be explored more to map regulation pathways, and further as to how it contributes to different neurodegenerative diseases. Understanding ADAR regulation and relation to disease pathology has the potential to open new perspectives in terms of how we understand and treat disease.

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1.2.3 ADAR3

ADAR3, like ADAR2, is mainly expressed neurologically – specifically in the amygdala and hypothalamus - however, unlike ADAR1 and ADAR2, ADAR3 is catalytically inactive Chen *et al.,* 2000). It is thought this catalytic inactivity is due to the fact that ADAR3 does not dimerise, in this way it is unable to form an active site (Wang *et al.,* 2019). However, this does not necessarily mean ADAR3 does not have a biological function. It has been proposed that ADAR3 regulates A-to-I editing through forming either a heterodimer with ADAR1 and ADAR2, or through binding to dsRNA at the appropriate site via the dsRBD. This has been observed several times, and therefore it is entirely plausible that ADAR3 acts as a negative regulator of ADAR1 and ADAR2 (Donnelly *et al.,* 2013; Oakes *et al.,* 2017). Whilst ADAR3 was not investigated in this project, just like ADAR1 and ADAR2 its potential for negative regulation opens up a possible pharmacological route in the treatment of diseases which show aberrant up-regulation of A-to-I editing.

1.3 Targeting RNA editing for therapy

In terms of disease causing mutations, RNA polymerase has a significantly higher error rate than DNA polymerase, mainly due to the fact that RNA polymerase lacks a proofreading mechanism (Chauhan, 2019). Recent researching have highlighted that aberrant RNA editing underlies many neurological and neurodevelopmental diseases, as well as having a significant role in many autoimmune disorders and different cancers (Eisenberg and Levanon, 2018; Gallo *et al.,* 2017; Scolding and Joseph, 2002; King *et al.,* 2016; Wang *et al.,* 2014).

The overview of the ADAR isoforms and their pivotal role in maintenance of the innate immune system, tumour suppression, and CNS functioning demonstrates how the regulation of ADAR editing could influence disease onset, treatment and prognosis. ADAR2 is critical for healthy neuronal development and maintenance, particularly when we see the case of maintaining calcium

impermeability of *GRIA2* (Sommer, 1991; Burnashev 1992; Lorneli 1994). Therefore, whilst we recognise other significant RNA editing events that occur throughout the body – and have identified molecules that appear to up-regulate RNA editing other than ADAR editing – we are particularly interested in molecules which up-regulate ADAR2 in the context of neurodegenerative disease. This project lays the foundation for further drug discovery projects and experiments, on which we can further investigate how exactly we can utilise pathways regulating ADAR to treat and understand disease further.

1.4 Structural Genomics Consortium Probes

In collaboration with a variety of pharmaceutical and academic partners, the Structural Genomic Consortium (SGC) generated a number of chemical probes to act as potent and selective inhibitors against epigenetic targets. They were designed to be used by biomedical researchers in order to explore and define the roles of different proteins, and begin early stages of drug discovery (https://www.thesgc.org/). We had access to 49 of these molecules (see table 1), as part of an ongoing collaboration, and sought to test those molecules for their ability to upregulate RNA editing activity by ADAR enzymes in neuronal cells.

2.0 MATERIALS AND METHODS

2.1 Reporter construct generation

The stable reporter cell line was constructed previously in the lab:

The design of the reporter construct was based on a previous study which used a sensitive ADAR editing reporter in cancer cells to screen a library of small molecules (Fritzell, Xu, Otrocka *et al.*, 2018). The stem-loop sequence included the R/G editing site from the GluA2 site within exon 11 – the most common site of editing in the healthy human brain (Fritzell, Xu, Otrocka *et al.*, 2018), it was synthesised with homologous ends to the target vector pCA923 under a pTDH3 promoter. The yeast pTHD3 promoter was replaced with a dox-inducible promoter allowing transient expression of the construct. A positive control was also created by site-directed mutagenesis of the editing site to a guanine, mimicking the effects of a permanent editing. A deletion of 18nt in the editing complementary sequence allowed the creation of a negative control, also. The reporter construct within the plasmid were amplified via polymerase chain reaction (PCR) with overhangs to a pLenti-puro plasmid (Addgene #39481). In this protocol we replaced firefly luciferase with mCherry, and nano-luciferase with eGFP (see figure 2).

The reporter-containing Lenti plasmid was co-transfected with the lentiviral envelope plasmid pVSVg (Addgene #8454) into Lenti-X 293T cell line. Lentiviral particles expressing the reporter cell lines were then harvested and transduced into mouse neuronal2A cells (n2A). n2A cells were grown in complete DMEM media with doxycycline, puromycin was added after 48hours to select for positive cells, FACS was also used to sort positive cells. Selected stable n2A cells were then cultured and passaged in completed DMEM growth medium.

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2.2 Cell Culture

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Adherent mouse Neuro 2A (N2A) cell lines were cultured in DMEM growth medium, supplemented with 10% FBS and 5% streptomycin at 37°C with 5% CO_2. When confluency reached 70% - 80%, as estimated by bright field microscopy, media was aspirated and cells were washed with 1X PBS (phosphate buffered saline pH 7.4, Thermo Fisher). Once PBS was removed, trypsin solution (Themo Fisher) was then added to the flask and incubated at 37°C for 3 minutes, or until cells were no longer adherent as seen by bright field microscopy. Fresh media was then added at a 1:1 ratio to inactivate the trypsin, aspirated and cells were pelleted by centrifugation at 1800 rpm for 6 minutes. Cells were then counted and seeded at a concentration of 1x10^6 cells/ml in a new 10ml culture flask.

2.3 Cryopreservation of cells and recovery of frozen cells

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To cryopreserve the cell line, cells were pelleted at 70%-80% confluency, as estimated by bright field microscopy, by centrifugation in 4°C at 1800rpm for 6 mins. The supernatant was then aspirated and the pellet was resuspended in freezing solution, which is constituted of 90% fetal bovine serum (FBS) and 10% dimethyl sulphoxide (DMSO; Sigma). A total of 1000ul was then transferred into cryopreservation vials , and slowly frozen down to -80°C in a cryo container (Nalgene® Mr. Frosty; Sigma). Cells were then stored at 80°C overnight before being transferred to liquid nitrogen storage.

In order to minimise exposure to DMSO, frozen cells were thawed quickly and re-suspended in 5 ml of fresh pre-warmed media. In order to remove DMSO, cells were then pelleted by centrifugation at 1800 rpm for 6 mins and resuspended in 10 ml complete medium. Cells were then grown in 25c m^3 flask at 37°C in a humidified incubator with 5% CO^2.

2.4 Cell counting

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Equal volume of cell suspension and Trypan Blue (Sigma) (1:1, 10ul each) were mixed. 10ul of solution was pipetted into either side of a haemocytometer, live cells were automatically counted using a cell counter (Biocompare) across four separate zones of the grid. Total concentration of cells (no. cells/ml of culture) was calculated by multiplying the average counted cells by multiplying the total volume of solution and the dilution factor of Trypan Blue. Results from either side of the haemocytometer were then averaged to provide an estimate of cell concentration.

2.5 Small molecules screening

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The library of small molecules was obtained from the Structural Genomic Consortium (SGC) (Williamoson AR, 2000). All the compounds, their targets and doses used in the study are listed in **Table 1**. Upon reception the molecules were diluted in DMSO to 10 mM (unless indicated otherwise), aliquoted and stored at -20°C for the duration of the study. Doxycycline was added to the cells at a final concentration of 1µg/ml to induce the expression of the reporter construct.

Table 1 – List of available SGC chemical probes

Small molecule	Target protein family	Specific Targets	Max. tolerated conc.
A-196	Methyltransferase	SUV420H1/H2	10 µM
A-366	Methyltransferase	G9a, GLP	10 µM
A-395	Methyl Lysine Binder	EED	10 µM
A-485	Acetyltransferase	p300, CBP	10 µM
BAY-299	Bromodomains	BRD1, TAF1	10 µM
BAY-598	Methyltransferase	SMYD2	10 µM
BAY-6035	Methyltransferase	SMYD3	10 µM
BAY-678	Serine Proteases	Neutrophil Elastase	10 µM
BAY-850	Bromodomains	ATAD2	2 µM
BAY-876	Glucose transporter	GLUT1	10 µM
BAZ2-ICR	Bromodomains	BAZ2A, BAZ2B	10 µM
BI-9564	Bromodomains	BRD9, BRD7	10 µM
BSP	Bromodomains	pan-Bromodomain	10 µM
GSK2801	Bromodomains	BAZ2A, BAZ2B	10 µM
GSK343	Methyltransferase	EZH2	10 µM

GSK4027	Bromodomains	PCAF, GCN5	10 μM
GSK484	Arginine deiminases	PAD-4	10 μM
GSK591	Methyltransferase	PRTM5	10 μM
GSK6853	Bromodomains	BRPF1	10 μM
GSK864	Dehydrogenase	Mutant isocitrate dehydrogenase 1	10 μM
GSK8814	Bromodomains	ATAD2, ATAD2B	10 μM
GSK-J1	Lysine Demethylase	JMJD3, UTX, JARID1B	10 μM
GSK-LSD	Lysine Demethylase	LSD1	10 μM
I-BRD9	Bromodomains	BRD9	10 μM
I-CBP112	Bromodomains	CREBBP, EP300	10 μM
IOX1	Lysine Demethylase	pan-2-OG	10 μM
IOX2	2OG	PHD2	10 μM
JQ1(+)	Bromodomains	BRD2, BRD3, BRD4, BRDT (BET)	10 μM
LLY-283	Methyltransferase	PRMT5	10 μM
L-MOSES	Bromodomains	PCAF bromodomain	10 μM
LP99	Bromodomains	BRD9, BRD7	10 μM
MS023	Methyltransferase	Type I PRMTs	10 μM
MS049	Methyltransferase	PRMT4,6	10 μM
NI-57	Bromodomains	BRPF1, BRPF2, BRPF3	10 μM
NVS-CECR2-1	Bromodomains	CECR2	1 μM
NVS-PAK1-1	Serine/threonine-protein kinase	PAK1	10 μM
OF-1	Bromodomains	BRPF1, BRPF2, BRPF3	10 μM
OICR-9424	WD40	WDR5	10 μM
PFI-1	Bromodomains	BRD2, BRD3, BRD4, BRDT (BET)	10 μM
PFI-2	Methyltransferase	SETD7	10 μM
PFI-3	Bromodomains	SMARCA,PB1	10 μM
PFI-4	Bromodomains	BRPF1B	10 μM
PFI-5	Methyltransferase	SMDY2	5 μM
SGC0946	Methyltransferase	DOT1L	10 μM
SGC3027	Methyltransferase	PRTM7	5 μM
SGC707	Methyltransferase	PRMT3	10 μM
SGC-CBP30	Bromodomains	CREBBP, EP300	10 μM
T-26c	Matrix metalloproteinase	MMP-13	10 μM
TP-064	Methyltransferase	PRMT4	10 μM
TP-472	Bromodomains	BRD9, BRD7	10 μM
UNC0638	Methyltransferase	G9a, GLP	2 μM
UNC0642	Methyltransferase	EHMT2 (G9a), EHTM1 (GLP)	2 μM
UNC1215	Methyl Lysine Binder	L3MBTL3	10 μM
UNC1999	Methyltransferase	EZH2	5 μM

2.6 Maximum tolerated concentration by cell lines.

MTS Cell Proliferation Assay Kit (Abcam) was performed by another member of the lab to determine the highest non-toxic concentration of small molecules in cells. Briefly, 3000 cells/wells were plated in triplicates in a 96-well plate in half of the final volume of medium (100 µL). After 6 h, compounds of interest were diluted to 2x final concentration in 100 µL of medium and added in a range of concentrations (1 µM to 10 µM). 1 µM of staurosporine (Sigma) was used as a positive control for cell death. After 48 h, 20 µL of MTS reagent was added to each well. Four hours later, the absorbance was measured at 490 nm on CLARIOstar plate reader (BMG Labtech). The concentration was deemed non-toxic if the absorbance was at least 80 % of the control absorbance and cells looked viable after visual inspection. In subsequent experiments, only the highest non-toxic concentration of each compound was used.

2.7 Fluorescence Screening of N2A cells and estimation of editing activity

N2A cells were plated in black 96-well plate (Corning) at a confluency of 1X10^5 cells/ml resuspended in complete medium with *doxycycline*. A 100 µl of cells were added across six wells per sample (10,000 cells per well). A hundred µl of the selected small molecule resuspended in complete media at 2X concentration was then added, creating a total volume of 200 µl per well. Cells were then incubated for 24 hours at 37°C in 5% CO_2. Following incubation, the media was aspirated from each well and replaced with 100 ul 1X PBS. The 96-well plate was then placed in the fluorescence reader (CLARIOstar, BMG Labtech). Peak optimisation was used to normalise the readings across the cells. Both *mCherry* and *eGFP* fluorescence was quantified. RNA editing activity was estimated by calculating *eGFP/mCherry* for each well of each sample, an average fold change was calculated across each sample and compared to the control sample of untreated *n2A* cells (average fold change of 1).

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2.8 RNA Extraction

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Cells were plated at a density of 1x10^6 cells/ml in a six well plate, three wells per treatment were used. Cells were plated with treatments as described above; cells were incubated for 24 hours before RNA extraction using RNeasy® Mini Kits (Qiagen). In summary, 350 µl buffer RLT was directly added to the well, followed by an equal volume of 70% ethanol. The solution was transferred to an RNAeasy spin column and centrifuged for 15 seconds at ≥8000 g, discarding the flow through. A total of 700 µl buffer RW1 was added to the spin column and centrifuged for 15 s at ≥8000 g, discarding the flow through, followed by 2 washed with buffer RPE. Lastly, the spin column was placed in a new 1.5ml collection tube, adding 30 µ of RNase-free water and centrifuged at ≥8000 g for the elution. Following extraction and purification of RNA I then measured the total concentration of RNA using a NanoDrop One® machine (Thermo Fischer) . I then added RNase-free water to each sample to create an equal concentration across all samples. Centrifuge was cooled to 4°C before extraction, samples were kept on ice at all times, and extracted and purified RNA was stored at -20°C.

2.9 cDNA Protocol

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Complementary DNA (cDNA) was generated using the Applied Biosystems™ High Capacity cDNA Reverse Transcription Kit (Thermo Fischer) protocol. A 10ul master mix (see table 2) was made and added to 10ul of purified RNA. Solution was vortexed and spun down before being added to the thermocycler. cDNA was stored at -20oC.

Table 2 – cDNA master mix component

Component	Volume/Reaction (µl)
10X RT Buffer	2.0
25X dNTP Mix (100nM)	0.8
10X RT Random Primers	2.0
Multiscribe™ Reverse Transcriptase	1.0
RNase Inhibitor	-

Nuclease-free H_2O	4.2
Total per Reaction	10.0

2.10 qRT-PCR Protocol

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Quantitative Reverse Transcription-PCR was carried out using the Sigma-Aldrich™ protocol and kit, a master mix was created using an adapted protocol (Table 3). A 18 µl master mix solution was added to 2 µl of sample cDNA, which was previously diluted to 100 µl in DNAse and RNase-free water. Three biological replicates of each sample were used. The plate was spun down and placed in a qPCR thermocycler (Abi StepOne plus). When using primers for the first time a melt curve was also performed to ensure adequate amplification. *Gapdh* was used as a reference gene.

Forward and reverse primers for the following targets: *Adar1*, *Adar2*, *Brd7* and *Gapdh* were designed and obtained from IDT (Integrated DNA Technologies™). Their sequences are indicated in Table 4.

Table 3 – qRT-qPCR multi-mix components

Component	**Volume/Reaction (ul)**
SYBR Green	10
Forward Primer (10uM stock)	0.9
Reverse Primer (10uM stock)	0.9
PCR grade water	6.2
Total per Reaction	18

Table 4 – Primer Sequences

Transcript name	Forward primer	Reverse primer
Adar1	CCTTCCCCATTCTCCACCTT	CCTTCCCCATTCTCCACCTT
Adar2	AGACATCCGAATCGCAAAGC	CCAGCGTGCTATCTTGTCAC
Brd7	AGCACAAGAAGCACAAGTCG	CATGGTCGCTTCTGTCTTCG
Gapdh	CACATCGCTCAGACACCATG	TGACGGTGCCATGGAATTTG

2.11 siRNA knockdown

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siRNAs specifically targeting Adar1, Adar2, or *Brd7* transcripts were purchased from Dharmacon. Twenty-four hours prior to transfection cells were seeded in a six well plate at a 60% confluency, as estimated by bright field microscopy. Lipofectamine® RNAiMAX (Thermo Fisher) reagent was diluted in Opti-MEM® Medium and incubated with 100 nM siRNAs also diluted in Opti-MEM® Medium for 25 min to allow for siRNA-lipid nanoparticle complexes to form. Transfected cells were visualised to ensure healthy growth and lack of toxicity.

2.12 Statistical Analysis

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Statistical analysis was carried out using Graphpad version 8, and significance was set as $p=<0.05$.

3.0 RESULTS

3.1 Generation of the editing reporter cell line

We aimed at generating a neuronal cell line stably expressing a reporter to monitor RNA editing activity that is sensitive to small fluctuations and has a quantitative read-out suitable for high-throughput measurements. A stem loop with the R/G editing site of the *GluA2* transcript modified to introduce an amber stop codon (UAG) that upon editing (UIG) is read as a tryptophan was cloned in between mCherry upstream, to enable internal normalisation, and eGFP downstream, to allow expression only upon editing of the stem loop (**Figure 2**) and inserted into the PiggyBac (PB) Transposon vector clone. This construct, which was already available in the lab, was engineered into neuronal2A cells using a piggyback backbone with an mCherry_T2_Glu2_STOP_eGFP_doxyclicine inducible construct and transposase. Puromycin selection was used to obtain monoclones, as well as FACS sorting, where they were then kept under doxycycline treatment in complete media. Clones expressing the highest levels of *mCherry* were further expanded and utilised for follow up experiments.

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Deleted: Neuro2a cells were engineered with a piggybac backbone with mCherry_ T2A_Glu2_STOP_eGFP_ doxocycline inducible construct and transposase. Monoclones were obtained under puromycin selection and FACS sorting. Cells were kept under doxocycline treatment in complete DMEM media¶

3.2 Screening of Chemical Probes

In order to identify small molecules epigenetic able to modulate RNA editing activity, we performed a cell-based screen in N2A cells carrying the *mCherry-stem loop-eGFP* transgene, using a collection of 52 chemical probes from the Structural Genomics Consortium (SGC, 2022) (**Table 1**). This unique library comprises compounds targeting epigenetic targets with high degree of potency and specificity (SGC, 2022). Doxycycline was added to cell media, at a 10mM concentration, in order to activate expression of the ADAR activity reporter. Cells were treated with each probe, diluted to their maximum tolerated concentration, which has been previously established by a viability assay. After 24hr incubation fluorescence of mCherry and eGFP were quantified, and fold change average of eGFP/mCherry, whereas >1 indicates increase and <1 a decrease in RNA editing activity, was calculated.

Of the 52 chemical probes, 5 were shown to significantly increase RNA editing activity (TP-472, NVS-CER2-1, BI-9564, GSK-864 and, MS-049) and 8 to decrease RNA editing activity (PFI-2, L-MOSES, BAY-299, OF-1, BAZ-2-ICR, A-485 and, GSK-591), as expressed by change in fluorescent fold change ($P \leq 0.05$, $n \geq 6$) **(Figure 3**). Out of the targets inhibited by these lists of small molecules, the greatest frequency of regulation of RNA editing was observed with probes acting upon the bromodomain (BRD) (TP-472, BI-9564, L-Moses and BAZ-2-ICR), and the methyltransferase (PRMT) (MS-049, GSK-591, PFI-2) family of proteins. The greatest increase in significant editing activity was observed upon treatment with MS049, a potent and selective inhibitor of PRMT4 and PRMT6, and with BRD7 inhibitors TP-472 and BI-9564 (**Figure 3**). The greatest decrease in significant editing activity was seen with GSK-591, a potent PRMT5 inhibitor, A485, a p300/CREB-binding protein acetyltransferase inhibitor, and BAZ2-ICR, BAY-299, L-MOSES and, OF-1, which target proteins of the bromodomain family (**Figure 3**).

A repeat screening of the 13 identified probes was carried out, further validating the results of the preliminary screening (**Figure 4**). Altogether, these results showed that basal RNA editing is amenable for epigenetic regulation and that both bromodomains and methyltransferases are promising targets for ADAR regulation.

3.3 qRT-PCR of Epigenetic Regulators of RNA editing activity

Given that RNA editing largely depends on ADAR1 and ADAR2 in mammalian cells, we next wondered whether the epigenetic regulators of RNA editing activity we identified affect ADAR protein expression. To answer this question, we treated N2A containing the dox-inducible *mCherry-stem loop-eGFP* construct with the validated regulators of RNA editing activity for 24-hours. Following the incubation, we extracted RNA from each sample ($n = 3$), and converted this to cDNA to carry out a qPCR to evaluate the expression of endogenous ADAR1 and ADAR2 in these cells (**Figure 5**).

Compared to untreated control, treatment with 11 epigenetic probes appeared to affect transcriptionally the expression of either ADAR 1, 2, or both. Transcriptional expression of ADAR1 was most significantly increased when cells were incubated with bromodomain inhibitor OF-1, whilst the greatest significantly decreased expression was seen when incubated with WD40 repeat inhibitor OICR-9429. For ADAR2 the greatest increase in transcriptional expression was seen in cells incubated with bromodomain inhibitor GSK-864, while the greatest decrease in transcriptional expression was seen in cells incubated with bromodomain inhibitor BAZ-2-ICR. Overall, dehydrogenase inhibitor GSK-864 appeared to lead to the greatest transcriptional up-regulation of both ADAR1 and ADAR2. GSK-864 inhibits IDH1, a gene encoding the enzyme isocitrate dehydrogenase 1, which regulates cellular redox homeostasis and has been linked to aberrant gene regulation in cancer, highlighting the role its inhibition plays in transcription control (Wang *et al.,* 2020).

3.4 Screening of epigenetic regulators of editing activity upon ADAR enzymes silencing

In order to assess whether the effects of epigenetic probes on RNA editing activity depends on transcriptional modulation of ADAR enzymes, we next performed the same fluorescence read-out upon siRNA-mediated knock-down of ADAR1 and ADAR2. In view of the scope and aims of this project, which is to identify small molecules able to boost RNA editing activity in mammalian cells, we decided to only move forward and further characterise the epigenetic up-regulators. First of all, we validated the efficiency of siRNA silencing of ADAR enzymes in cells. Treatment of N2A with 100nM siRNA specifically targeting mouse *Adar1* or *Adar2* successfully determined silencing of the targeted gene (**Figure 6**). We next proceeded to perform the treatments with epigenetic up-regulators of editing activity upon knock-down of *Adar1*, *Adar2*. As expected there was a significant decrease in editing when both *Adar1* and *Adar2* were silenced, holding up with the current understanding that ADAR is responsible for the majority of endogenous editing that occurs in cells. Both *Adar1* and *Adar2* knockdown counteracted the effects of bromodomain inhibitors GSK-864 and

BI-564, PRMT inhibitor MS-049, and methyltransferase inhibitor PFI-2. Interestingly only *Adar2* knockdown led to a significant decrease in editing in TP-472, whilst *Adar1* knockdown was not significant.

3.5 Bromodomain 7 and ADAR activity

Having established the expression of which ADAR enzyme (ADAR1 or ADAR2) is crucial for the effect of the identified editing regulators, we next sought to understand the exact mechanism of action. When deciding on how to tackle this question we looked at the class of proteins each probe inhibited as well as their individual target (**Table 5**). Most of the identified small molecule regulators of editing activity belong to the family of bromodomain inhibitors (BAY-299, BAZ-2-ICR, BI-9564, TP-472 and, OF-1). Additionally, TP-472 and BI-9564 treatment led to a transcriptional increase in *Adar1* and *Adar2* expression in N2A. Whilst both TP-472 and BI-9564 are shown to inhibit both Brd7 and Brd9, I-Brd9 inhibits only Brd9 and no significant change in endogenous RNA editing activity was observed with this small molecule. Therefore, we hypothesised that it is likely that Brd7 and not Brd9 that specifically controls transcriptionally ADAR expression. Brd7 is a known regulator of transcriptional activity, acting both as a co-activator and a co-repressor (Liu *et al.,* 2016). We knocked down *Brd7* transcript in N2A cells by siRNA and verified that upon silencing of *Brd7*, both *Adar1* and *Adar2* transcripts increase by almost 2 fold (**Figure 7**), suggesting that this protein exerts negative transcriptional control over ADAR enzyme expression. Altogether these results suggest that Brd7 inhibition using epigenetic small molecules increase basal RNA editing activity by increasing the expression of ADAR enzymes.

4.0 Discussion

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4.1 Methyltransferase inhibition leads to transcriptional down-regulation of ADAR1/2

Of the identified regulators of RNA editing activity, three were protein arginine N-methyltransferases (PRMT) inhibitors: MS049, which inhibits PRMT4 and PRMT6, and PFI-2 which inhibits SET-domain containing lysinemethyltransferase-7 (SETD7), both of which appeared to up-regulate RNA editing of the reporter system; and GSK-591 which inhibits PRMT5, and appeared to down regulate RNA editing of the reporter system. Interestingly all of these probes appeared to transcriptionally down-regulate *Adar1 and Adar2* transcripts (**figure 5).**

PRMTs are a family of proteins promoting methylation arginine residues in histone and non-histone proteins (Wei, Mundade, Lange and Lu, 2013). The type of methylation promoted by PRMT is either: monomethylation (MMA), asymmetric (ADMA), or symmetric di-methylation (SDMA) (Bedford, Mark and Clarke, 2009). One of the effects of methylation is to affect transcription by altering the recruitment of transcription factors (Bedford, Mark and Clarke, 2009). CREB (cAMP response element-binding protein) is a transcription factor, it regulates diverse cellular transcriptional responses by interacting with CRE (cAMP response element), a specific sequence found around promoter sites of genes (Wen, Sakamoto and Miller, 2010). Genome wide screening has revealed that over 4000 genes are regulated by CRE (Zhang *et al.,* 2005). CREB is recruited through the transcriptional co-activators p160 and CBP/p300, PRMT4 regulates these co-activators by

Table 5 – SGC Probes Inhibitory targets

Class	Probe	Target
Bromodomain	TP-472	Brd7/9
	BI-9564	Brd7/9
Dehydrodgenase	GSK-864	IDH1
Methyltransferase	MS-049	PRMT4/PRMT6
	PFI2	SETD7

methylating and binding (Modak *et al.,* 2013; Blanco *et al.,* 1998). By binding to these co-activators

there is increased methylation of target genes, enhancing the accessibility of promoter regions for transcription (Wang *et al.,* 2018; Wen, Sakamoto and Mller, 2010). Therefore, inhibition of PRMT4 by MS-049 (**Table 5**) would lead to a decrease in the recruitment of CREB, and as such down-regulate transcription. Furthermore, PRMT4 directly methylates core histones H3R17 and H3R26 – key epigenetic transcriptional activation marks (Holmvist and Mannervik, 2013; Hatanake *et al.,* 2017), therefore inhibition of PRMT4 would also reduce chromatin remodelling, decreasing transcription. Interestingly, the decrease in CREB recruitment leads to an increased pool of CBP, which is able to regulate transcription through steroid hormone receptors (Beato, Chavez and Truss, 1996). PRMT6 is another inhibitory target of MS-049, however PRMT6 specifically mediates asymmetric demethylation of H3R2 – a marker for transcriptional repression (Tewary, Zheng and Ho, 2019). Therefore, inhibition of PRMT6 would result in an increase in transcription around H3R2. Considering these contrasting mechanisms of action, we feel the best way to explain the decrease in *Adar* expression (**Figure 5**) is due to PRMT4 inhibition rather than PRMT6. However, it is important to note that whilst *Adar* appeared to be down-regulated, there was an increase in editing of the reporter site observed; this could mean that PRMT6 inhibition led to the up-regulation of other RNA editing mechanisms, or the increase in CBP may up-regulate steroid-activated transcription which again up-regulates other RNA editing mechanisms.

PFI-2 inhibits SETD7, this protein methylates H3K4 – a marker of transcriptional activation, it also recruits transcription factors directly to histones (see table 5) (Blanco *et al.,* 1998). Therefore it would be expected that inhibition of SETD7 would lead to transcriptional repression due to a reduction in methylation of H3K4, as well as a decrease in transcription factor recruitment. Again, this fits with the reduction in *Adar1* and *Adar2* transcripts observed upon incubation; however there is still an overall increase in RNA editing of the reporter molecule.

GSK-591 inhibits PRMT5, and whilst RNA editing of the reporter construct was reduced the reduction of *Adar1* and *Adar 2* was not significant **(figure 2, figure 5)**. PRMT5 dimethylates H4R3/8 and H2AR,

generally PRMT5 methylation of targets results in transcriptional repression (Batista and Helguero, 2018; Deng *et al.,* 2017). Therefore, it would be expected that PRMT5 inhibition by GSK-591 would result in an up-regulation of transcription. PRMT 5 targets histone 2 and histone 4, it does not appear play a significant role in transcription regulation as its counterparts PRMT4 and SETD7 **(Figure 8)** (Batista and Helguero, 2018; Liu *et al.,* 2020), therefore this could suggest that it is the involvement of transcriptional factors and co-activators which effect *Adar* transcription rather than direct histone acetylation. However, further studies would be needed to confirm this.

Overall, it is clear that inhibition of methyltransferases reduces expression of ADAR, however in some cases lead to increase in RNA editing events. Considering the key regulatory role methylation plays in chromatin remodelling it is not unsurprising that we see such a mixed effect, however further studies would be needed to further understand these mechanisms. This clearly highlights how these targets could be utilised pharmacologically to regulate RNA editing events.

4.2 Mutant dehydrogenase as a target for editing activity

GSK-864 inhibits mutant dehydrogenase IDH1 inhibitor, and appeared to show a significant increase in editing activity as well as the greatest significant increase in both *Adar1* and *Adar2* transcription. Wild-type IDH1 forms a homodimer in the cytoplasm to catalyse the reversible oxidative decarboxylation of isocitrate to a-ketoglutarate (a-KG) reaction within the Krebs cycle (Dimitrov *et al.,* 2015; Xu *et al.,* 2004). However, mutant IDH1s undergo an amino acid substitution of the active site in codon 132, the change in binding site activity results in a loss of function and production of 2-hydroxyglutarate (2-HG) rather than a-KG (Turkalp *et al.,* 2014; Liu Ling, 2015; Buscar, 2018). 2-HG has been shown to activate the transcription factor hypoxia-inducible factor 1α (HIF1-a), leading to the transcriptional up-regulation of HIF1-a inducible genes (Williams 2022). This has been shown to lead to an up-regulated *CD24* expression, which has been associated with histone acetylation (Mimeault and Batra, 2014; Kown *et al.,*2015). A transcriptome analysis revealed that in $CD24^{+}$ cell populations expressing mutant IDH1 that there were 285 upregulated genes and 293 downregulated

genes, compared to $CD24^-$ cells expressing mutant IDH1 (Kown *et al.,* 2015; Turcan *et al.,* 2018). ChIP-seq analysis of the major histone modifications of $CD24^+$ cells showed a positive correlation between CD24 expression and H3k4me3 – associated with transcriptional activation – and H3K9me3 and H4k20me3 (marks of transcriptional repression) (Turcan *et al.,* 2017). Considering this we would expect inhibition of mutant IDH1 to results in a mix of transcriptional up-regulation and down-regulation, clearly inhibition of mutant IDH1 leads to a clear transcriptional increase of both *Adar1* and *Adar2*, as well as an increase in RNA editing of the reporter site. Whilst further studies are required to deduce the exact mechanism behind this, mutant IDH1, as well as GSK-864, appear to be strong contenders for further studies into uncovering the pathway behind ADAR regulation. Mutant IDH1 has been associated with human malignancies (Turcan, 2018), therefore pharmacologically the inhibition of mutant IDH1 may offer a possible therapy for the treatment of malignancies to slow or inhibit growth. Furthermore, a Hilbert curve analysis suggests that H3K9me3 and H3K4me3 (marks of transcriptional repression and activation, respectively) are not spread evenly across the genome, but rather around 5'UTR and promoter regions (Anders, 2009; Turcan *et al.,* 2017). A Hilbert-curve is a space-filling curve which covers the entirety of the genome and therefore can be used to plot locations of residues. These results again add to the pharmacological attractiveness of mutant IDH1 as a target, and GSK-864 as a treatment, as the localisation of results around promoter regions and 5'UTR make the transcriptional effects more predictable.

Overall, mutant IDH1 has a very broad regulatory effect, both for transcriptional activation and repression; inhibition clearly improves A-to-I editing efficiency, and as such is a clear regulator of ADAR activity. Whilst further studies are required to observe and measure the full effect on cellular metabolism and viability from mutant IDH1 inhibition, it does present itself as an attractive and intriguing target for further study.

4.3 Interference with the PCAF and BAF domains decreases transcription of ADAR1/2

Of the five identified up-regulators of RNA editing activity, four of these appear to act on converging mechanisms of transcriptional regulation (**Figure 8**). MS-049 inhibits PRMT4, which is involved in the formation, regulation, and activity of PCAF (p300/CBP-associated factor) – a mammalian transcriptional coactivator, and member of the GNAT family (Kiernan *et al.,*1999). PCAF interacts with CBP (CREB binding protein) and p300 (Szyf, 2009; Modak *et al.,* 2013). PCAF regulates transcriptional activity through histone acetylation, particularly H314K and H4K8 and other non-histone proteins e.g. p53 (Blanco *et al.,* 1998; Wang, Qui and Wu, 2018). Considering PCAF is widely considered a transcriptional coactivator (Xenaki *et al.,* 2008), it is therefore not surprising that a decrease in ADAR1/2 transcription is observed upon treatment with an epigenetic inhibitor, despite an overall increase in RNA editing activity, suggesting that mechanisms other than ADAR transcriptional regulation play a role in mediating this effect.

The bromodomain forms a four a-helices bundle which is suggested to act as a chromatin targeting modules (Winston and Allis, 1999), they are able to specifically bind acetylated chromatin and facilitate chromatin remodelling and modification through association with transcription factors including PCAF (Dhalluin *et al.,* 1999; Hudson *et al.,* 2000; Jacobson *et al.,* 2000; Owen *et al.,* 2000; Cong *et al.,* 2005). Bromodomains have also been shown to transcriptionally regulate signalling molecules, and in yeast, HATs (histone acetyltransferases), which lack bromodomains have been shown to result in a number of significant transcriptional related defects (Marcus *et al.,* 1994; Dyson, Rose and Madhevan, 2001), highlighting the significant role bromodomains play in transcription. Bromodomain-containing protein 7 (BRD7) is a component of one form of the SWI/SNF chromatin remodelling complex and has been shown to have a specific affinity for acetylated histones. Knock-down experiments have indicated that the binding of BRD7 to histone 3 is the underlying mechanism of the transcriptional role on target genes (Cong *et al.,* 2005). As explained in the results section, we

deduced the editing and transcriptional effect observed upon incubation of TP-472 and BI-9564 is caused through inhibition of BRD7. BRD7, by binding to acetylated histones, in particular histone 3, is able to guide transcription factors and signalling molecules towards target genes. Interestingly, as suggested by Kaeser *et al.,* (2008), acetylation of histone 3 by BRD7 leads to recruitment of transcription factors such as SWI/SNF (BAF) complex and PCAF. Whilst histone acetylation typically enhances transcription, and deacetylation represses transcription, the reverse has also been observed – in which histone acetylation led to a repression in transcription (Raemon-Buettner and Borlak, 2007; Damodaran, 2011). This would explain why inhibition of BRD7 via treatment with TP-472 and BI-9564 led to an increase in ADAR1/2 transcription.

4.4 Bromodomain 7 is a key regulator of ADAR1/2 transcription

The observations of an increase in ADAR expression on incubation of TP-472 and BI-9564 led us to hypothesise that BRD7 is a key regulator of ADAR expression. This hypothesis appeared to be confirmed in our knock-down experiment of BRD7, when knockdown of BRD7 expression led to a 1.7x increase in transcriptional expression of both ADAR1 and ADAR2. Whilst further studies would be required to further investigate the precise mechanism of this regulation, our results suggest a strong and significant transcriptional correlation between BRD7 expressions and ADAR expression, hence RNA editing activity.

4.5 Non-transcriptional control of RNA editing activity

One interesting finding from our reporter system, is that the two ADAR enzymes, ADAR1 and ADAR2, contribute to baseline editing in N2A cells to a different extent and with different responsiveness upon treatments, suggesting different mechanisms of regulation. ADAR1 knock-down led to a decrease in editing below that of the untreated control in GSK-864, MS-049 and BI-9564, whilst

ADAR2 knock-down only led to a decrease in editing activity below the untreated control when treated with PFI-2. However, across all treatments other than TP-472, knockdown of ADAR1 and 2 both led to a significant reduction in editing activity as measured by mean fold change of fluorescence. This can lead us to confirm that ADAR is responsible for the majority of RNA editing observed across the identified up-regulators.

Whilst TP-472 seemed to lead to an increase in transcription of ADAR and overall editing activity, upon ADAR1/2 knockdown there was still an overall gross increase in editing activity compared to an untreated control, suggesting other mechanisms of regulation, which are independent from the transcriptional regulation of ADAR1 and ADAR2.

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5.0 Conclusions and limitations of the study

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Here we have screened a list of highly potent and selective epigenetic small molecules for their ability to modulate endogenous RNA editing activity in neuronal cell lines, using the mCherry-stem_loop-eGFP fluorescent reporter system in a neuronal cell line. The identified regulators of RNA editing activity require further investigation as to their effect on 'real' RNA editing target assessed by RNA-sequencing, and require testing within disease model cell-lines and animals in order to conclusively evaluate their potential in treating disease. Nevertheless, we believe we have provided here preliminary evidence of the identification of RNA editing modulation by epigenetic small molecule regulators, which could be taken further for drug discovery. As well as this, we have also identified BRD7 as a key regulator of ADAR transcriptional activity – likely through the PCAF domain, as well as highlighting the potential key role that methyltransferase inhibition has on regulating editing activity also. Mutant IDH1 has also presented itself as an interesting target, the inhibition of which appeared to have the greatest increase in ADAR transcription and up-regulation of editing activity. We have also observed that common to many of our chemical probes inhibitory targets, in which there was a significant change in ADAR transcription compared to the control, was Histone 3 targeting: This included PRMT4, inhibited by MS-049; SETD7, inhibited by PFI-2; mutant IDH1, inhibited by GSK-864; and PCAF – inhibited by TP-472, BI-9564, and MS-049.

This study screened a library of small molecules by incubating n2A cells containing the reporter construct at their maximum tolerated concentration, as deduced by cell assay. It is therefore important to consider that the identified compounds may have broader effects which contribute to the results. A further experiment that should be performed is a dose-dependent experiment to identify the lowest dose at which we observe an increase in editing, RNA-seq analysis could also be done to identify any off-target effects. Further to this, whilst a translational effect of editing is inferred due to increase in eGFP:mCherry fluorescence, we were only able to confirm this

transcriptionally via qPCR. Western blot, or proteomic studies should be used in further studies to confirm that the increased transcription of ADAR is also observed translationally.

We sought to identify molecules that alter RNA editing activity, our reporter construct was designed to be a specific editing site for ADAR, therefore our results are only useful in identifying molecules that effect ADAR RNA editing. However, whilst ADAR editing is the main mechanism of RNA editing within mammals, there are other mechanisms and targets of RNA editing which we were unable to address here. Again, an RNA-seq analysis would be useful in analysing additional RNA editing effects of these molecules.

Unfortunately, whilst we were able to identify TP-472 as being a likely up-regulator of RNA editing activity, and having a similar mechanism of action as potential recruiters of ADAR due to a lack of material we were unable to use it in further experiments.

Overall, while further work needs to be done to understand the exact mechanism of action of lead candidates, this project has gone some way as to exploring how different classes of protein families contribute to regulation of RNA editing, divulging potential protein pathways of transcriptional regulation. Considering the potential that the control of RNA editing activity by ADAR enzymes offers in the treatment and prevention of disease, this project has laid some important groundwork on which to build upon in further studies when it comes to understanding what pathways are involved in regulation of RNA editing, and how can we utilise those pathways when treating diseases, where aberrant RNA editing occurs.

6.0 Figures and Legends

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Figure 1

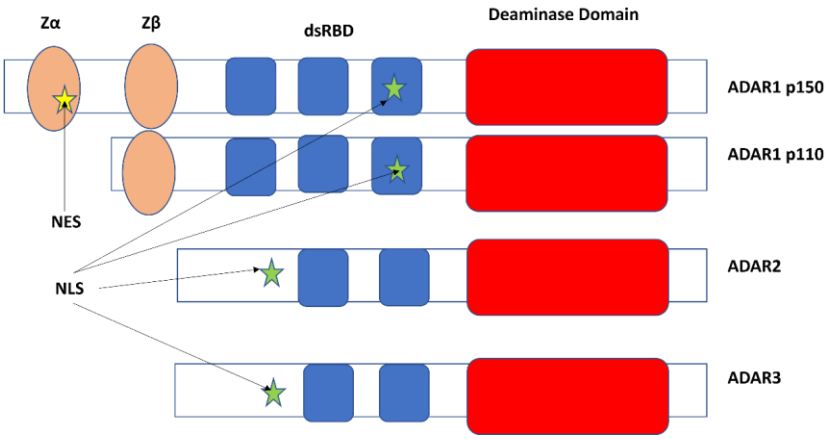

Fig 1. Schematic illustration of ADAR isoforms.

ADAR1 occurs as two main isoforms due to alternate promotor sites: ADAR1p150, involved in innate immunity, contains a deaminase domain and three dsRBDs – common to ADAR1, however ADARp150 has a n-terminal extension containing two Z-DNA binding domains. The Zα-domain contains a nuclear export signal (NES) which allows ADARp150 to enter the cytosol in order to carry out it's immune functions. ADAR1p110 is localised within the nucleus due to the nuclear localisation signal (NLS) in the first dsRBD. Unlike ADAR1p150, ADARp110 lacks the Zα-domain and as such is unable to enter the cytosol. ADAR2 contains a c-terminal deaminase domain with two dsRBDs, it also contains an n-terminal NLS. ADAR3 has a seminal structure to ADAR2, however it is unable to dimerise and as such is catalytically inactive (Sakurai *et al.*, 2017).

Figure 2

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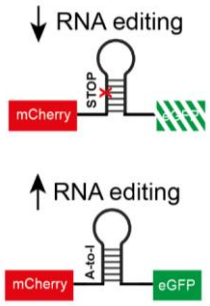

Fig 2. Schematic illustration of the *mCherry-stem_loop-eGFP* construct

A stem loop editing of the GluA2 R/G editing site transcripts being read as an amber stop codon (UAG), which upon editing is read as tryptophan (UIG). This construct was inserted into the PiggyBac(PB) Transposon vector clone and transfected Neuro 2A (n2A) cells.

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Figure 3

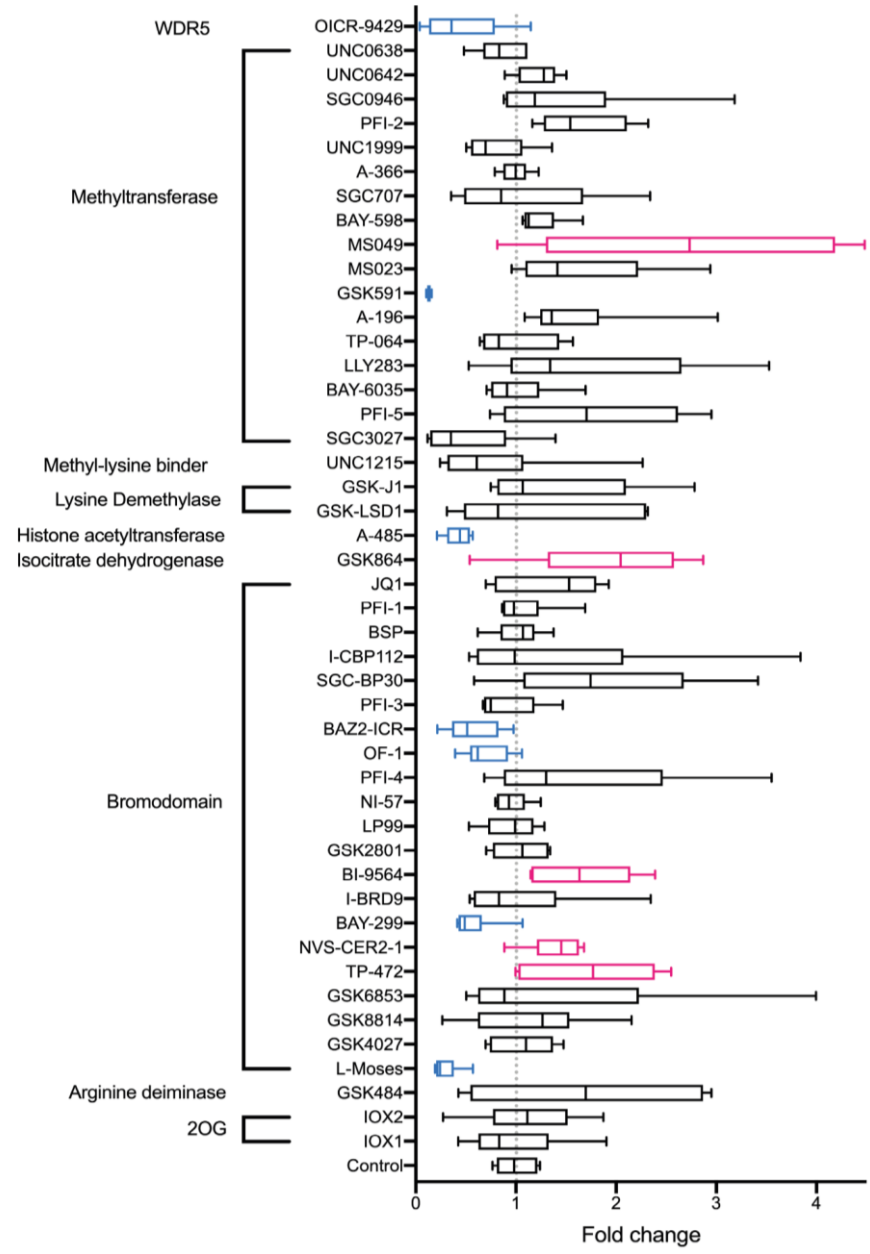

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Fig 3. Preliminary screening for epigenetic regulators of RNA editing

We had 47 chemical probes inhibiting 9 protein families, we incubated n2A cells containing the dox-inducible *mCherry-stem_loop-eGFP* construct (see figure 1) at the tolerated maximum concentrations for 24 hours in a 92-well plate (see table 1). A two-tailed paired t-test was compared testing each probe against untreated control cells (n=6), standard error of the mean was also included. Following 24-hours incubation media was aspirated from each well and replaced with 100uM of PBS solution, before being loaded into the plate reader for fluorescence quantification. The ratio of *eGFP:mCherry* was calculated and compared to the control to produce a fold change. Six probes were identified which appeared to increase RNA editing activity compared to the control at significant levels (p=<0.05), shown in pink: PFI-2 and MS-049 (methyltransferase inhibitors), GSK-864 (isocitrate dehydrogenase inhibitor), BI-9564, NVS-CER2-1 and TP472 (bromodomain inhibitors). Seven of the probes appeared to down-regulate RNA editing at a significant level (p=<0.05), shown in blue: OICR-9429 (WDR5 inhibitor), A-485 (histone acetyltransferase inhibitor); BAZ2-ICR, OF-1, BAY-299, OF-1 and, L-Moses (bromodomain inhibitor). For both identified up-regulators and identified down-regulators most probes were concentrated as bromodomain inhibitors, however the greatest increase in editing activity was seen by MS-049, a methyltransferase inhibitor, and the greatest decrease in editing activity was seen upon incubation with GSK-591 – also a methyltransferase inhibitor.

Figure 4

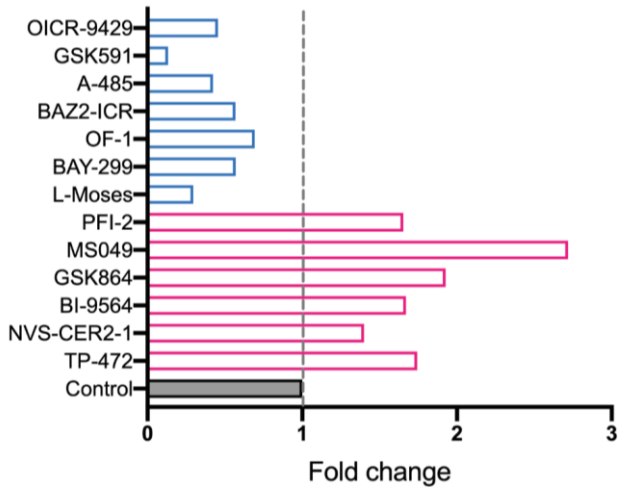

Fig 4. Repeat fluorescence screening of significant regulators of RNA editing activity.

Chemical probes were incubated with cells at the maximum tolerated concentration (n=6) on a 96-well dish, expression of the *mCherry-stem_loop-eGFP* was induced with incubation with doxycycline. Fluoresence ratio of *eGFP:mCherry* was quantified after 24 hour incubation to confirm significant results observed in the preliminary screening.). A two-tailed paired t-test was compared testing each probe against untreated control cells (n=6), with a significance of p=<0.05 across across all probes apart from NVS-CER2-1 which we excluded from further experiments.

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We had 47 chemical probes inhibiting 9 protein families, we incubated n2A cells containing the dox-inducible *mCherry-stem_loop-eGFP* construct (see figure 1) at the tolerated maximum concentrations for 24 hours in a 92-well plate (see table 1). A two-tailed paired t-test was compared testing each probe against untreated control cells (n=6), standard error of the mean was also included. Following 24-hours incubation media was aspirated from each well and replaced with 100uM of PBS solution, before being loaded into the plate reader for fluorescence quantification. The ratio of *eGFP:mCherry* was calculated and compared to the control to produce a fold change. Six probes were identified which appeared to increase RNA editing activity compared to the control at significant levels (p=<0.05), shown in pink: PFI-2 and MS-049 (methyltransferase inhibitors), GSK-864 (isocitrate dehydrogenase inhibitor), BI-9564, NVS-CER2-1 and TP472 (bromodomain inhibitors). Seven of the probes appeared to down-regulate RNA editing at a significant level (p=<0.05), shown in blue: OICR-9429 (WDR5 inhibitor), A-485 (histone acetyltransferase inhibitor); BAZ2-ICR, OF-1, BAY-299, OF-1 and, L-Moses (bromodomain inhibitor). For both identified up-regulators and identified down-regulators most probes were concentrated as bromodomain inhibitors, however the greatest increase in editing activity was seen by MS-049, a methyltransferase inhibitor, and the greatest decrease in editing activity was seen upon incubation with GSK-591 – also a methyltransferase inhibitor.

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Figure 5

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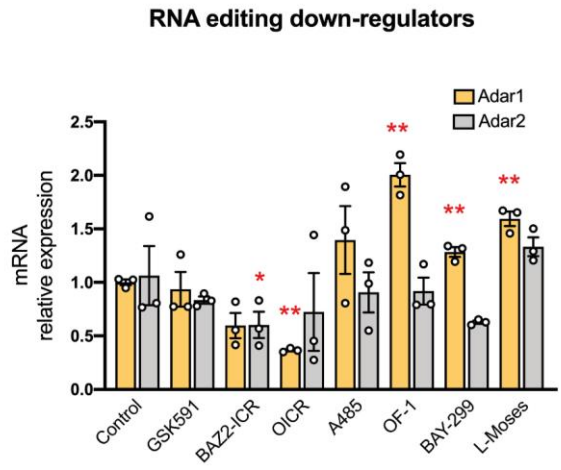

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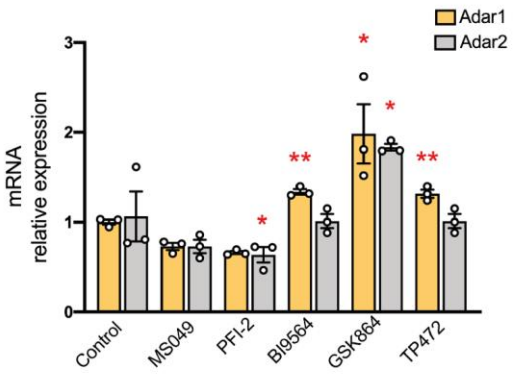

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Fig 5. Effect of identified regulators of RNA editing activity of ADAR1 and ADAR2 transcription.

RT-qPCR analysis of ADAR1/2 expression across n2A cells treated with identified up and down regulators of RNA editing activity in order to see whether these epigentic regulators also effect ADAR expression. n2A cells were treated with the identified regulators of RNA editing activity at their maximum tolerated concentration (n=3), as well as doxycycline to induce expression of the *mCherry-stem_loop-eGFP* construct, and incubated for 24-hours. Statistical analysis was carried out using a calculation of the standard error of the mean and a two-tailed paired t-test, *p=<0.05, **p=<0.01.

a) Incubation with five probes led to a significant change in the expression of ADAR1 or ADAR2: ADAR1 was significantly increased upon incubation with OF-1**, BAY-299** and L-Moses**. ADAR 1 was significantly decreased OICR-9429** and whilst being decreased when incubated with other probes, these were not significant. ADAR2 was not significantly increased. Or decreased, upon incubation with any of the identified down-regulators of RNA editing activity.

b) Within the up-regulators both ADAR 1 and ADAR2 expression were both significantly increased in GSK-864* incubated cells. ADAR 1 was significantly increased in cells incubated with BI-9564** and TP-472**, with no significant decrease or increase observed in cells incubated with other significant up-regulators of RNA editing activity. ADAR2 was significantly down-regulated when incubated with PFI-2*, with no other significant increase or decrease apart from cells incubated with GSK-864*.

Moved down [2]: **Fig 5.** Effect of identified regulators of RNA editing activity of ADAR1 and ADAR2 transcription.¶
RT-qPCR analysis of ADAR1/2 expression across n2A cells treated with identified up and down regulators of RNA editing activity in order to see whether these epigentic regulators also effect ADAR expression. n2A cells were treated with the identified regulators of RNA editing activity at their maximum tolerated concentration (n=3), as well as doxycycline to induce expression of the *mCherry-stem_loop-eGFP* construct, and incubated for 24-hours. Statistical analysis was carried out using a calculation of the standard error of the mean and a two-tailed paired t-test, *p=<0.05, **p=<0.01.¶
a) Incubation with five probes led to a significant change in the expression of ADAR1 or ADAR2: ADAR1 was significantly increased upon incubation with OF-1**, BAY-299** and L-Moses**. ADAR 1 was significantly decreased OICR-9429** and whilst being decreased when incubated with other probes, these were not significant. ADAR2 was not significantly increased. Or decreased, upon incubation with any of the identified down-regulators of RNA editing activity. ¶
b) Within the up-regulators both ADAR 1 and ADAR2 expression were both significantly increased in GSK-864* incubated cells. ADAR 1 was significantly increased in cells incubated with BI-9564** and TP-472**, with no significant decrease or increase observed in cells incubated with other significant up-regulators of RNA editing activity. ADAR2 was significantly down-regulated when incubated with PFI-2*, with no other significant increase or decrease apart from cells incubated with GSK-864*.

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Figure 6

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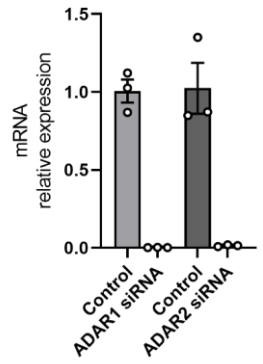

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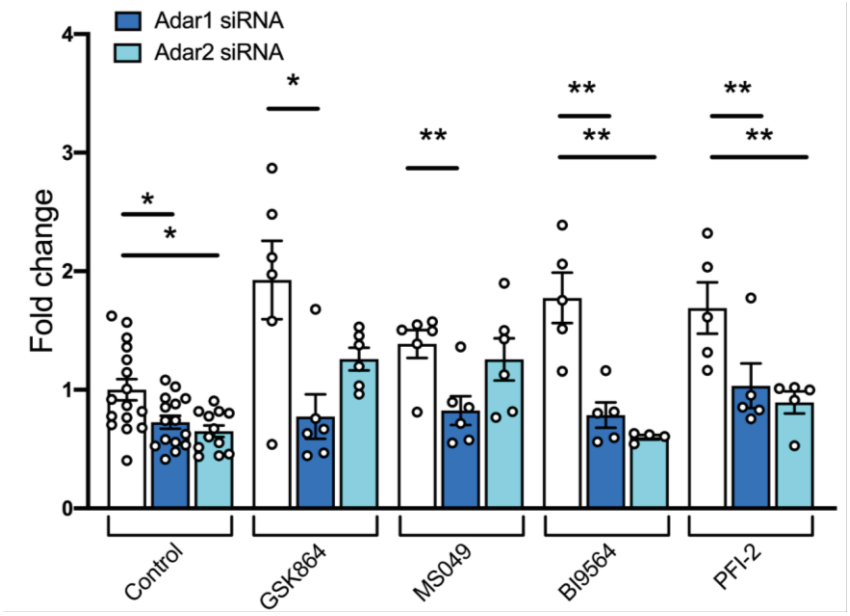
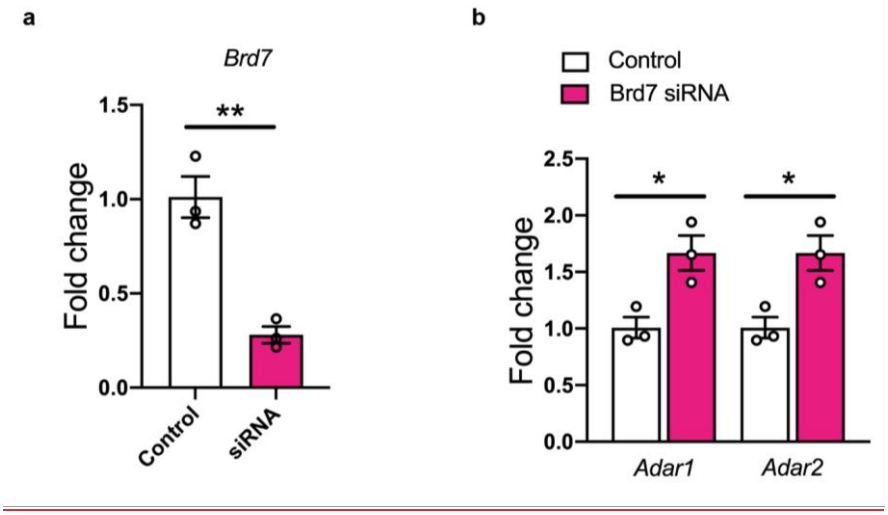


Fig 6. Quantification of contribution of ADAR 1 and ADAR 2 to RNA editing activty of identified up-regulators.

n2A cells were treated with 100nM ADAR1 and ADAR2 siRNA for 24 hours, and then treated with identified epigenetic regulators of RNA editing activity, as well as 10mM *dox* to induce expression of the *eGFP:mCherry* construct. Knock-down cells were transferred to a 96 well plate before being treated with the identified regulators of RNA activity. Following 24-hours incubation media was aspirated from each well and replaced with 100uM of PBS solution, before being loaded into the plate reader for fluorescence quantification. By comparing how knockdown of ADAR1 and ADAR2 effects the *eGFP:mCherry* ratio, we are able to visualise how far ADAR 1 and ADAR 2 contribute individually and together to the change in RNA editing seen upon incubation with these probes. Compared to untreated controls we are able to see that all wild-type cells show an increase in RNA editing activity when treated with each identified up-regulator of RNA editing activity, with GSK-864(IDH1 inhibitor) showing the greatest increase in editing activity (c.x1.25), followed by TP-472 (a BRD7/9 inhibitor), PFI2, BI-9564 and MS049. ADAR 2 knockdown appeared to reduce editing activity the most in PFI-2 (a SETD7 methyltransferase inhibitor), TP-472 and BI-9564 (BRD7/9 bromodomain inhibitors), ADAR1 knockdown also led to a significant reduction in editing activity, with reduction almost as great as in ADAR 2 knockdown. Interestingly, ADAR 1 knockdown led to almost a twice as great reduction in RNA editing activity in n2A cells treated with GSK-864 (an IDH1, dehydrogenase inhibitor) and MS-049 (a PRMT4/6, methyltransferase inhibitor).

Additionally, we also knocked down ADAR1 and ADAR2 in untreated n2A cells in order to compare how ADAR 1 and ADAR 2 individually contribute to endogenous editing. Both results were significant, with ADAR2 leading to a 34% reduction in endogenous editing activity (p=<0.05), and ADAR 1 knockdown leading to a 27% reduction in endogenous editing activity (p=<0.05).

Figure 7



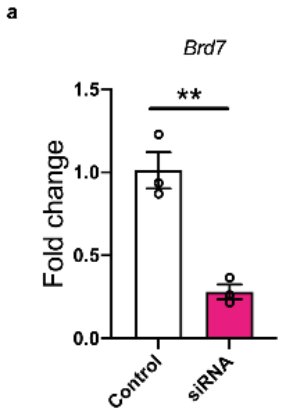


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Fig. 7 Brd7 knockdown effect on ADAR 1 and ADAR 2 expression in n2A cells.

A two-tailed paired t-test, and standard error the mean, was calculated in order to identify significant results (p=<0.05*, p=<0.01**).

a) Bromodomain 7 knockdown was optimised in n2A cells at a siRNA concentration of 500nM**. qRT-PCR was carried out following 24 hours incubation with siRNA to quantify the transcriptional expression of ADAR 1 and ADAR 2.
b) Knockdown of Brd7 at 500nM led to a significant increase in both ADAR 1 and ADAR 2*, being x1.6 greater than in wild type control n2A cells. These results indicate that Brd7 may be a key protein in the regulation of ADAR 1 and ADAR 2 expression.

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Figure 8

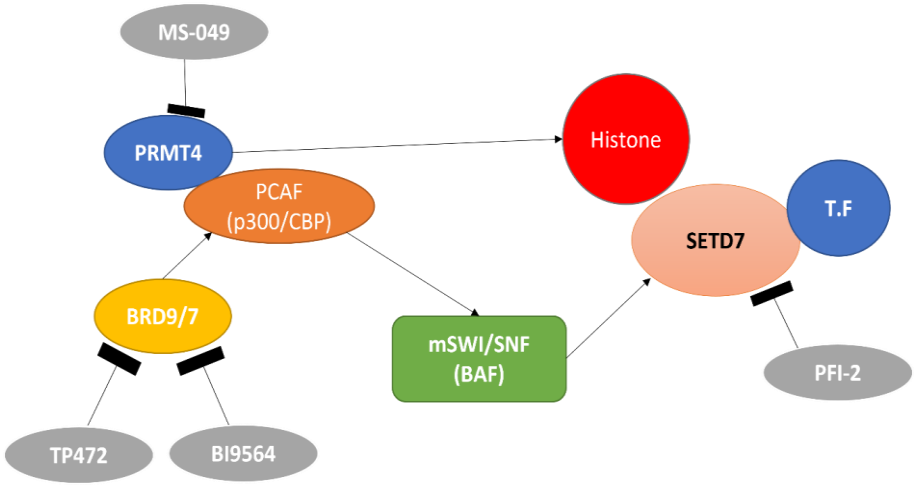

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Fig 8. Schematic representation of how identified chemical probes interfere with the transcriptional pathway.

This diagram highlights how four of the identified up-regulators of RNA editing activity interact within the transcriptional pathway. MS-049 inhibits PRMT4 from binding with the PCAF complex, a chromatin remodelling factor – recruiting SETD7. In this way chromatin remodelling is effected, interfering with transcription. PRMT4 also directly acetylates histones, in this inhibition of PRMT4 directly effects the state of histones. Both TP-472 and BI-9564 inhibit Brd7/9, Brd7 and Brd9 effect the formation of the PCAF complex, and as such inhibition of Brd7/9 leads to decreased recruitment of the BAF complex, affecting chromatin remodelling. PFI-2 inhibit SETD7, a histone methyltransferase, SETD7 is known to recruit transcription factors to the histone through methylation of histones. Inhibition of SETD7 will aalso effect chromatin remodelling, and as such transcription (Kiernan *et al.,* 1999; Szyf, 2009; Blanco *et al.,* 1998).

Bibliography

Aizawa H, Hideyama T, Yamashita T, Kimura T, Suzuki N, Aoki M, Kwak S (2016) 'Deficient RNA-editing enzyme ADAR2 in an amyotrophic lateral sclerosis patient with a FUS(P525L) mutation'. *J Clin Neurosci* 32:128–129. doi:10.1016/j.jocn.2015.12.039

Bascur, Juan P.; Alegria-Arcos, Melissa; Araya-Duran, Ingrid; Juritz, Ezequiel I.; Gonzalez-Nilo, Fernando D.; Almonacid, Daniel E. (2018). "IDH1 and IDH2 mutants identified in cancer lose inhibition by isocitrate because of a change in their binding sites". *bioRxiv*: 425025. doi:10.1101/425025.

Basilio C, Wahba AJ, Lengyel P, Speyer JF, Ochoa S (1962) Synthetic polynucleotides and the amino acid code. Proc Natl Acad Sci USA 48:613–616

Basilio C, Wahba AJ, Lengyel P, Speyer JF, Ochoa S. Synthetic polynucleotides and the amino acid code. *V Proc Natl Acad Sci USA.* (1962) 48:613–6. 10.1073/pnas.48.4.613

Batista, I. and Helguero, L., (2018). 'Biological processes and signal transduction pathways regulated by the protein methyltransferase SETD7 and their significance in cancer'. *Signal Transduction and Targeted Therapy*, 3(1).

Beato, M., Chaves, S., and Truss M. (1996) 'Transcriptional regulation by steroid hormones.' *Steroids*, 61(4), pp.240-251

Bedford, M. and Clarke, S.G. (2009) 'Protein arginine and methylation in mammals: who, what, and why'. *Molecular cell* 33(1)

Ben-Shlomo H., Levitan A., Shay N.E., Goncharov I., Michaeli S., (1999). 'RNA editing associated with the generation of two distinct conformations of the trypanosomatid Leptomonas collosoma 7SL RNA'. *J. Biol. Chem.* 274, pp: 25642 – 50

Blanco, J.C.; Minucci, S.; Lu, J.; Yang, X.J.; Walker, K.K.; Chen, H.; Evans, R.M.; Nakatani, Y.; Ozato, K. (1998) 'The histone acetylase PCAF is a nuclear receptor coactivator'. *Genes Dev. 12*, pp: 1638–1651.

Bycroft M, Grunert S, Murzin AG, Proctor M, St Johnston D (1995) NMR solution structure of a dsRNA binding domain from *Drosophila* staufen protein reveals homology to the N-terminal domain of ribosomal protein S5. EMBO J 14:3563–3571

Chen CX, Cho DS, Wang Q, Lai F, Carter KC, Nishikura K (2000) A third member of the RNA-specific adenosine deaminase gene family, ADAR3, contains both single- and double-stranded RNA binding domains. *RNA* 6 pp: 755–767

Chen L, Li Y, Lin CH, Chan THM, Chow RKK, Song Y, Liu M, Yuan YF, Fu L, Kong KL, Qi L, Li Y, Zhang N, Tong AHY, Kwong DLW, Man K, Lo CM, Lok S, Tenen DG, & Guan XY (2013). Recoding RNA editing of AZIN1 predisposes to hepatocellular carcinoma. *Nature Medicine*, 19(2), 209–216. 10.1038/nm.3043

CHEN, C., CHO, D., WANG, Q., LAI, F., CARTER, K. and NISHIKURA, K., 2000. A third member of the RNA-specific adenosine deaminase gene family, ADAR3, contains both single- and double-stranded RNA binding domains. *RNA*, 6(5), pp.755-767.

Chuahan T. (2019). 'DNA polymerase synthesises the DNA while RNA polymerase synthesies the RNA' Genetic Education Inc. Comparison Between: DNA Polymerase Vs RNA Polymerase (geneticeducation.co.in) Accessed: 22/9/2022

Crow, Y. J. (2011) 'Type I interferonopathies: a novel set of inborn errors of immunity'. *Ann. N. Y. Acad. Sci.* 1238, 91–98.

Crow, Y. J. et al. (2006). 'Mutations in the gene encoding the 3'-5' DNA exonuclease TREX1 cause Aicardi-Goutieres syndrome at the AGS1 locus'. *Nat. Genet.* 38, 917–920.

Dacwag C. S., Bedford M. T., Sif S., Imbalzano A. N. (2009). 'Distinct protein arginine methyltransferases promote ATP-dependent chromatin remodeling function at different stages of skeletal muscle differentiation'. *Mol. Cell. Biol.* 29 1909–1921. Doi: 10.1128/mcb.00742-08

d'Angelo DM, Di Filippo P, Breda L, Chiarelli F. (2021). 'Type I Interferonopathies in Children: An Overview'. *Front Pediatr*. 9:631329.. doi:10.3389/fped.2021.631329

Deng X, Shao G, Zhang HT, Li C, Zhang D, Cheng L, Elzey BD, Pili R, Ratliff TL, Huang J, Hu CD. Protein arginine methyltransferase 5 functions as an epigenetic activator of the androgen receptor to promote prostate cancer cell growth. Oncogene. 2017 Mar 2;36(9):1223-1231. doi: 10.1038/onc.2016.287

Desterro JMP, Keegan LP, Lafarga M, Berciano MT, O'Connell M, Carmo-Fonseca M (2003) Dynamic association of RNA-editing enzymes with the nucleolus. J Cell Sci 116:1805–1818. doi:10.1242/jcs.00371

Dhalluin C, Carlson JE, Zeng L, He C, Aggarwal AK, Zhou M-M. (1999). 'Structure and ligand of a histone acetyltransferase bromodomain'. *Nature* 399: 491– 496.

Dhir A, Dhir S, Borowski LS, Jimenez L, Teitell M, Rotig A, et al. (2018). Mitochondrial double-stranded RNA triggers antiviral signalling in humans. *Nature.* 560:238–42. 10.1038/s41586-018-0363-0

Dimitrov L, Hong CS, Yang C, Zhuang Z, Heiss JD (2015). "New developments in the pathogenesis and therapeutic targeting of the IDH1 mutation in glioma". *International Journal of Medical Sciences*. 12 (3): 201–13. Doi:10.7150/ijms.11047

Donnelly CJ, Zhang P-W, Pham JT, Haeusler AR, Mistry NA, Vidensky S, Daley EL, Poth EM, Hoover B, Fines DM (2013) RNA toxicity from the ALS/FTD C9ORF72 expansion is mitigated by antisense intervention. *Neuron* 80 pp: 415–428

Dyson M., Rose S., and Louis C. (2001) 'ACETYLLYSINE-BINDING AND FUNCTION OF BROMODOMAIN-CONTAINING PROTEINS IN CHROMATIN'. *Frontiers in Bioscience* 6, pp: 853-865

Eggington JM, Greene T, Bass BL (2011) Predicting sites of ADAR editing in double-stranded RNA. Nat Commun 2:319. doi:10.1038/ncomms1324

Evans AJ, Gurung S, Wilkinson KA, Stephens DJ, Henley JM (2017) 'Assembly, secretory pathway trafficking, and surface delivery of kainate receptors is regulated by neuronal activity'. *Cell Rep* 19:2613–2626

Fritzell K., Xu L. , Otrocka M., Andréasson C., Öhman M. (2018), 'Sensitive ADAR editing reporter in cancer cells enables high-throughput screening of small molecule libraries' *Nucleic Acids Research*, Volume 47, Issue 4, Page e22, https://doi.org/10.1093/nar/gky1228

Gallo, A. and Locatelli, F., (2011). ADARs: allies or enemies? The importance of A-to-I RNA editing in human disease: from cancer to HIV-1. *Biological Reviews*, 87(1), pp.95-110.

Gallo, A., Vukic, D., Michalík, D. *et al.* (2017). 'ADAR RNA editing in human disease; more to it than meets the I'. *Hum Genet* 136, 1265–1278. https://doi.org/10.1007/s00439-017-1837-0

George CX, & Samuel CE (1999). Human RNA-specific adenosine deaminase ADAR1 transcripts possess alternative exon 1 structures that initiate from different promoters, one constitutively active and the other interferon inducible. *Proceedings of the National Academy of Sciences of the United States of America*, 96(8), 4621–4626. 10.1073/pnas.96.8.4621

Goodman RA, Macbeth MR, Beal PA. (2012). ADAR proteins: structure and catalytic mechanism. *Curr Top Microbiol Immunol*.;353:1-33. doi: 10.1007/82_2011_144. PMID: 21769729.

Gordon Betts J.,, Young K., Wise J.A, Johnson E., Poe B., Kruse D.H., Korol O., Johnson J.E., Womble M., DeSaix P. (2013). 'Anatomy and Physiology', OpenStax, Texas

Greenblatt S., Man N., Hamard P-J., Martinez C., Xu Y.,, Liu F., Watts J.M., Tenen D.G., Nimer S.D. (2017). 'CARM1/PRMT4 As a Novel Therapeutic Target for AML'. *Blood* 130 (1) 241. doi: https://doi.org/10.1182/blood.V130.Suppl_1.241.241

Gumireddy K, Li A, Kossenkov AV, Sakurai M, Yan J, Li Y, Xu H, Wang J, Zhang PJ, Zhang L, Showe LC, Nishikura K, & Huang Q (2016). The mRNA-edited form of GABRA3 suppresses GABRA3-mediated Akt activation and breast cancer metastasis. *Nature Communications*, 7(1), 1–9. 10.1038/ncomms10715

Gunawardena, R., Fox, S., Siddiqui, H. and Knudsen, E., 2007. SWI/SNF Activity Is Required for the Repression of Deoxyribonucleotide Triphosphate Metabolic Enzymes via the Recruitment of mSin3B. *Journal of Biological Chemistry*, 282(28), pp.20116-20123.

Hatanaka, Y., Tsusaka, T., Shimizu, N., Morita, K., Suzuki, T., Machida, S., Satoh, M., Honda, A., Hirose, M., Kamimura, S., Ogonuki, N., Nakamura, T., Inoue, K., Hosoi, Y., Dohmae, N., Nakano, T., Kurumizaka, H., Matsumoto, K., Shinkai, Y. and Ogura, A., (2017). 'Histone H3 Methylated at Arginine 17 Is Essential for Reprogramming the Paternal Genome in Zygotes'. *Cell Reports*, 20(12), pp.2756-2765.

Heale, B. S. E., Keegan, L. P., & O'Connell, M. A. (2010). The effect of RNA editing and ADARs on miRNA biogenesis and function. *Advances in Experimental Medicine and Biology*, *700*, 76-84.

Hideyama T, Yamashita T, Aizawa H, Tsuji S, Kakita A, Takahashi H, Kwak S (2012) 'Profound downregulation of the RNA editing enzyme ADAR2 in ALS spinal motor neurons'. *Neurobiol Dis* 45:1121–1128. doi:10.1016/j.nbd.2011.12.033

Higuchi M, Maas S, Single F, Hartner J, Rozov A, Burnashev N, Feldmeyer D, Sprengel R, Seeburg P (2000) 'Point mutation in an AMPA receptor gene rescues lethality in mice deficient in the RNA-editing enzyme ADAR2'. *Nature* 406:78–81

Higuchi M, Single FN, Kohler M, Sommer B, Sprengel R, Seeburg PH (1993) RNA editing of AMPA receptor subunit GluR-B: a base-paired intron-exon structure determines position and efficiency. Cell 75:1361–1370

Honoré T, Lauridsen J, Krogsgaard-Larsen P (1982). "The binding of [3H]AMPA, a structural analogue of glutamic acid, to rat brain membranes". *Journal of Neurochemistry*. 38 (1): 173–8.

Hu X, Chen J, Shi X, Feng F, Lau KW, Chen Y, Chen Y, Jiang L, Cui F, Zhang Y, Xu X, & Li J (2017). RNA editing of AZIN1 induces the malignant progression of non-small-cell lung cancers. *Tumor Biology*, 39(8), 101042831770000. 10.1177/1010428317700001

Hudson BP, Martinez-Yamout MA, Dyson HJ, Wright PE. (2000). 'Solution structure and acetyl-lysine binding activity of the GCN5 bromodomain'. *J Mol Biol* 304: 355– 370.

Jacobson RH, Ladurner AG, King DS, Tjian R. (2000). 'Structure and function of a human TAFII250 double bromodomain module'. *Science* 288 pp: 1422– 1425.

Jonatha M. Gott Ronald B. Emeson (2000). 'FUNCTIONS AND MECHANISMS OF RNA EDITING' *Annual Review of Genetics* 34(1), pp: 499-531

Kaeser MD, Aslanian A, Dong MQ, Yates JR 3rd, Emerson BM. (2008). 'BRD7, a novel PBAF-specific SWI/SNF subunit, is required for target gene activation and repression in embryonic stem cells'. *The Journal of Biological Chemistry*. 283(47) pp: 32254-32263. DOI: 10.1074/jbc.m806061200

Kawahara Y, *et al. (2003)* 'Low editing efficiency of GluR2 mRNA is associated with a low relative abundance of ADAR2 mRNA in white matter of normal human brain' *Eur. J. Neurosci*., 18, pp. 23-33

Kawahara Y., *et al.(2004)* **'**Glutamate receptors: RNA editing and death of motor neurons' *Nature*, 427, p. 801

Keegan, L., Gallo, A. and O'Connell, M., 2001. The many roles of an RNA editor. *Nature Reviews Genetics*, 2(11), pp.869-878.

Kiernan, R. E., Vanhulle, C., Schiltz, L., Adam, E., Xiao, H., Maudoux, F., Calomme, C., Burny, A., Nakatani, Y., Jeang, K. T., Benkirane, M., and Van Lint, C. (1999) HIV-1 tat transcriptional activity is regulated by acetylation EMBO J. 18, 6106– 6118

Kim YK, Furic L, DesGroseillers L, & Maquat LE (2005). Mammalian Staufen1 recruits Upf1 to specific mRNA 3'UTRs so as to elicit mRNA decay. *Cell*, 120(2), 195–208. 10.1016/j.cell.2004.11.050

King AE, Woodhouse A, Kirkcaldie MT, Vickers JC (2016) Excitotoxicity in ALS: overstimulation, or overreaction? *Exp Neurol* 275(Pt 1):162–171. doi:10.1016/j.expneurol.2015.09.019

Kuttan, A. and Bass, B., 2012. Mechanistic insights into editing-site specificity of ADARs. *Proceedings of the National Academy of Sciences*, 109(48).

Kwon, M., Han, J., Seo, J., Song, K., Jeong, H., Choi, J., Kim, Y., Lee, S., Choi, Y. and Shin, Y.,(2015). 'CD24 Overexpression Is Associated with Poor Prognosis in Luminal A and Triple-Negative Breast Cancer'. *PLOS ONE*, 10(10), p.e0139112.

Lamers MM, van den Hoogen BG, Haagmans BL. (2019). ADAR1: "Editor-in-Chief" of Cytoplasmic Innate Immunity. *Front Immunol*;10:1763. doi:10.3389/fimmu.2019.01763

Lebon, P. et al. (1988). 'Intrathecal synthesis of interferon-alpha in infants with progressive familial encephalopathy'. *J. Neurol. Sci.* 84, 201–208

Lehmann KA, Bass BL (2000) Double-stranded RNA adenosine deaminases ADAR1 and ADAR2 have overlapping specificities. *Biochemistry* 39:12875–12884

Lemmens, R., Moore, M., Al-Chalabi, A., Brown, R. and Robberecht, W., 2010. RNA metabolism and the pathogenesis of motor neuron diseases. *Trends in Neurosciences*, 33(5), pp.249-258.

Levanon, E., Eisenberg, E., Yelin, R., Nemzer, S., Hallegger, M., Shemesh, R., Fligelman, Z., Shoshan, A., Pollock, S., Sztybel, D., Olshansky, M., Rechavi, G. and Jantsch, M., 2004. Systematic identification of abundant A-to-I editing sites in the human transcriptome. *Nature Biotechnology*, 22(8), pp.1001-1005.

Licht K, Kapoor U, Amman F, Picardi E, Martin D, Bajad P, & Jantsch MF (2019). A high resolution A-to-I editing map in the mouse identifies editing events controlled by pre-mRNA splicing. *Genome Research*, 29(9), 1453–1463. 10.1101/gr.242636.118

Liu F, Xu Y, Lu X, Hamard PJ, Karl DL, Man N, Mookhtiar AK, Martinez C, Lossos IS, Sun J, Nimer SD. (2020) 'PRMT5-mediated histone arginine methylation antagonizes transcriptional repression by polycomb complex PRC2'. *Nucleic Acids Res*. 48(6) pp: 2956-2968. doi: 10.1093/nar/gkaa065

Liu X, Ling ZQ (2015). "Role of isocitrate dehydrogenase 1/2 (IDH 1/2) gene mutations in human tumors". *Histology and Histopathology*. 30 (10): 1155–60. doi:10.14670/HH-11-643.

Livingston JH, Lin JP, Dale RC, Gill D, Brogan P, Munnich A, Kurian MA, Gonzalez-Martinez V, De Goede CG, Falconer A, Forte G, Jenkinson EM, Kasher PR, Szynkiewicz M, Rice GI, Crow YJ (2014) A type I interferon signature identifies bilateral striatal necrosis due to mutations in ADAR1. J Med Genet 51:76–82. doi:10.1136/jmedgenet-2013-102038

Maas, S. and Gommans, W., 2009. Identification of a selective nuclear import signal in adenosine deaminases acting on RNA. *Nucleic Acids Research*, 37(17), pp.5822-5829.

Macbeth MR, Schubert HL, VanDemark AF, Lingam AT, Hill CP, Bass BL (2005) Structural biology: inositol hexakisphosphate is bound in the ADAR2 core and required for RNA editing. Science 309:1534–1539. doi:10.1126/science.1113150

Marcus G. A, Silverman N., Berger S.L., Horiuchi J. and, Guarente L. (1994). 'Functional similarity and physical association between GCN5 and ADA2: putative transcriptional adapters'. *EMBO J* 13, pp: 4807-4815

Masquida B, Westhof E. On the wobble GoU and related pairs. *RNA.* (2000) 6:9–15. 10.1017/S1355838200992082

Matthews MM, Thomas JM, Zheng Y, Tran K, Phelps KJ, Scott AI, Havel J, Fisher AJ, Beal PA (2016) Structures of human ADAR2 bound to dsRNA reveal base-flipping mechanism and basis for site selectivity. Nat Struct Mol Biol 23:426–433. doi:10.1038/nsmb.3203

Michlewski G, & Cáceres JF (2019). Post-transcriptional control of miRNA biogenesis. *RNA*, 25(1), 1–16. 10.1261/rna.068692.118

Mimeault, M. & Batra, S. K. (2014). 'Molecular biomarkers of cancer stem/progenitor cells associated with progression, metastases, and treatment resistance of aggressive cancers'. *Cancer Epidemiol. Biomarkers Prev*. 23, pp: 234–254.

Mittaz, L., Antonarakis, S., Higuchi, M. and Scott, H., 1997. Localization of a novel human RNA-editing deaminase (hRED2 or ADARB2) to chromosome 10p15. *Human Genetics*, 100(3-4), pp.398-400.

Moshe Szyf, 'CHAPTER 5 – Epigenomics and Its Implications for Medicine', Editor(s): Huntington F. Willard, Ph.D., Geoffrey S. Ginsburg, *Genomic and Personalized Medicine,* Academic Press, 2009, Pages 60-73, ISBN 9780123694201, https://doi.org/10.1016/B978-0-12-369420-1.00005-6.

Nishikura K. Functions and regulation of RNA editing by ADAR deaminases. *Annu Rev Biochem.* (2010) 79:321–49. 10.1146/annurev-biochem-060208-105251

Oakes E, Anderson A, Cohen-Gadol A, Hundley HA (2017) Adenosine deaminase that acts on RNA 3 (ADAR3) binding to glutamate receptor subunit B pre-mRNA inhibits RNA editing in glioblastoma. *J Biol Chem* 292:4326–4335

Ota H, Sakurai M, Gupta R, Valente L, Wulff BE, Ariyoshi K, Iizasa H, Davuluri RV, & Nishikura K (2013). ADAR1 forms a complex with dicer to promote microRNA processing and RNA-induced gene silencing. *Cell*, 153(3), 575–589. 10.1016/j.cell.2013.03.024

Oudhoff MJ, Freeman SA, Couzens AL, Antignano F, Kuznetsova E, Min PH, Northrop JP, Lehnertz B, Barsyte-Lovejoy D, Vedadi M, Arrowsmith CH, Nishina H, Gold MR, Rossi FM, Gingras AC, Zaph C. (2013). 'Control of the hippo pathway by Set7-dependent methylation of Yap'. *Dev Cell*. 29;26(2):188-94. Doi: 10.1016/j.devcel.2013.05.025

Owen DJ, Ornaghi P, Yang JC, Lowe N, Evans PR, Ballario P. (2000). 'Functional organization of the yeast SAGA complex: Distinct components involved in structural integrity, nucleosome acetylation, and TATA-binding protein interaction'. *EMBO J* 19 pp: 6141– 6149.

Patterson, J. and Samuel, C., 1995. Expression and regulation by interferon of a double-stranded-RNA-specific adenosine deaminase from human cells: evidence for two forms of the deaminase. *Molecular and Cellular Biology*, 15(10), pp.5376-5388.

Peng C., ,Zhou J., ,Liu H., Ying,Z., Ming,W.L., Li,Z., Qiu H, Yang Y., Xin X., , Wei,S Shou R, Li Xiao L, Li Gui Y (2005). 'The transcriptional regulation role of BRD7 by binding to acetylated histone through bromodomain' *Journal of Cellular Biochemistry'* 97(4) pp:882-892

Peng Y, Croce CM. (2016). The role of MicroRNAs in human cancer. Signal Transduct Target Ther. 28(1) pp:15004. doi: 10.1038/sigtrans.2015.4. PMID: 29263891; PMCID: PMC5661652.

Peng, P., Zhong, X., Tu, W., Soundarapandian, M., Molner, P., Zhu, D., Lau, L., Liu, S., Liu, F. and Lu, Y., (2006). ADAR2-Dependent RNA Editing of AMPA Receptor Subunit GluR2 Determines Vulnerability of Neurons in Forebrain Ischemia. *Neuron*, 49(5), pp.719-733.

Per-Henrik Holmqvist & Mattias Mannervik (2013) 'Genomic occupancy of the transcriptional co-activators p300 and CBP', *Transcription*, 4(1), 18-23, DOI: 10.4161/trns.22601

Poulsen, H., Jorgensen, R., Heding, A., Nielsen, F., Bonven, B. and Egebjerg, J., 2006. Dimerization of ADAR2 is mediated by the double-stranded RNA binding domain. *RNA*, 12(7), pp.1350-1360.

Poulsen, H., Nilsson, J., Damgaard, C., Egebjerg, J. and Kjems, J., 2001. CRM1 Mediates the Export of ADAR1 through a Nuclear Export Signal within the Z-DNA Binding Domain. *Molecular and Cellular Biology*, 21(22), pp.7862-7871.

Qin YR, Qiao JJ, Chan THM, Zhu YH, Li FF, Liu H, Fei J, Li Y, Guan XY, & Chen L (2014). Adenosine-to-inosine RNA editing mediated by ADARs in esophageal squamous cell carcinoma. *Cancer Research*, 74(3), 840–851. 10.1158/0008-5472.CAN-13-2545

Rahul Modak, Jeelan Basha, Narendra Bharathy, Koustav Maity, Pushpak Mizar, Akshay V. Bhat, Madavan Vasudevan, Vinay Kumar Rao, Wai Kay Kok, Nagashayana Natesh, Reshma Taneja, and Tapas K. Kundu (2013) 'Probing p300/CBP Associated Factor (PCAF)-Dependent Pathways with a Small Molecule Inhibitor'. *ACS Chemical Biology 8* (6), pp: 1311-1323 DOI: 10.1021/cb4000597

Rodero MP, & Crow YJ (2016). Type I interferon-mediated monogenic autoinflammation: The type I interferonopathies, a conceptual overview. *Journal of Experimental Medicine*, 213(12), 2527–2538. 10.1084/jem.20161596

Roth SH, Danan-Gotthold M, Ben-Izhak M, Rechavi G, Cohen CJ, Louzoun Y, & Levanon EY (2018). Increased RNA editing may provide a source for autoantigens in systemic lupus erythematosus. *Cell Reports*, 23(1), 50–57. 10.1016/j.celrep.2018.03.036

S. Kwak, *et al.* (2008) 'Newly identified ADAR-mediated A-to-I editing positions as a tool for ALS research'*RNA Biol*., 5, pp. 193-197

S.T. Reamon-Buettner, J. Borlak (2007). 'A new paradigm in toxicology and teratology: altering gene activity in the absence of DNA sequence variation' *Reprod Toxicol*, 24, pp. 20-30

Sakurai M, Shiromoto Y, Ota H, Song C, Kossenkov AV, Wickramasinghe J, Showe LC, Skordalakes E, Tang HY, Speicher DW, & Nishikura K (2017). ADAR1 controls apoptosis of stressed cells by inhibiting

Staufen1-mediated mRNA decay. *Nature Structural and Molecular Biology*, 24(6), 534–543. 10.1038/nsmb.3403

Samuel CE. (2019). Adenosine deaminase acting on RNA (ADAR1), a suppressor of double-stranded RNA-triggered innate immune responses. *J Biol Chem.*294:1710–20. 10.1074/jbc.TM118.004166

Scolding NJ, Joseph FG. (2002). The neuropathology and pathogenesis of systemic lupus erythematosus*. Neuropathol Appl Neurobiol*. 28(3):173-89. doi: 10.1046/j.1365-2990.2002.00406.x.

Shigeyasu K, Okugawa Y, Toden S, Miyoshi J, Toiyama Y, Nagasaka T, Takahashi N, Kusunoki M, Takayama T, Yamada Y, Fujiwara T, Chen L, & Goel A (2018). AZIN1 RNA editing confers cancer stemness and enhances oncogenic potential in colorectal cancer. *JCI Insight*, 3(12), 1–14. 10.1172/jci.insight.99976

Shiromoto Y, Sakurai M, Qu H, Kossenkov AV, & Nishikura K (2020). Processing of Alu small RNAs by DICER/ADAR1 complexes and their RNAi targets. *RNA*, 26, rna.076745.120. 10.1261/rna.076745.120

Sinigaglia K, Wiatrek D, Khan A, Michalik D, Sambrani N, Sedmík J, Vukić D, O'Connell MA, Keegan LP. (2018) 'ADAR RNA editing in innate immune response phasing, in circadian clocks and in sleep' *Biochim Biophys Acta Gene Regul Mech*. 1862(3):356-369. doi: 10.1016/j.bbagrm.2018.10.011.

Sommer B, Köhler M, Sprengel R, Seeburg PH (1991) 'RNA editing in brain controls a determinant of ion flow in glutamate-gated channels'. *Cell* 67:11–19

Song B, Shiromoto Y, Minakuchi M, Nishikura K. (2022) The role of RNA editing enzyme ADAR1 in human disease. *Wiley Interdiscip Rev RNA*;13(1):e1665. doi:10.1002/wrna.1665

St Johnston D, Brown NH, Gall JG, Jantsch M (1992) A conserved double-stranded RNA-binding domain. Proc Natl Acad Sci USA 89:10979–10983

Stefl R, Oberstrass FC, Hood JL, Jourdan M, Zimmermann M, Skrisovska L, Maris C, Peng L, Hofr C, Emeson RB, Allain FH (2010) The solution structure of the ADAR2 dsRBM-RNA complex reveals a sequence-specific readout of the minor groove. Cell 143:225–237

Takeuchi O, Akira S. Pattern recognition receptors and inflammation. *Cell.* (2010) 140:805–20. 10.1016/j.cell.2010.01.022

Teoh PJ, An O, Chung TH, Chooi JY, Toh SHM, Fan S, Wang W, Koh BTH, Fullwood MJ, Ooi MG, de Mel S, Soekojo CY, Chen L, Ng SB, Yang H, & Chng WJ (2018). Aberrant hyperediting of the myeloma transcriptome by ADAR1 confers oncogenicity and is a marker of poor prognosis. *Blood*, 132(12), 1304–1317. 10.1182/blood-2018-02-832576

Tewary SK, Zheng YG, Ho MC. (2019) 'Protein arginine methyltransferases: insights into the enzyme structure and mechanism at the atomic level'. *Cell Mol Life Sci.* 76(15) pp:2917-2932. doi: 10.1007/s00018-019-03145-x

Tirupapuliyur V. Damodaran, 'Chapter 71 - Genotoxicities and infertility', Editor(s): Ramesh C. Gupta, *Reproductive and Developmental Toxicology*, Academic Press, 2011, Pages 923-947, ISBN 9780123820327, https://doi.org/10.1016/B978-0-12-382032-7.10071-2.

Tomaselli, S., Bonamassa, B., Alisi, A., Nobili, V., Locatelli, F. and Gallo, A., 2013. ADAR Enzyme and miRNA Story: A Nucleotide that Can Make the Difference. *International Journal of Molecular Sciences*, 14(11), pp.22796-22816.

Turcan S, Makarov V, Taranda J, Wang Y, Fabius AWM, Wu W, Zheng Y, El-Amine N, Haddock S, Nanjangud G, LeKaye HC, Brennan C, Cross J, Huse JT, Kelleher NL, Osten P, Thompson CB, Chan TA.

Mutant-IDH1-dependent chromatin state reprogramming, reversibility, and persistence. Nat Genet. 2018 Jan;50(1):62-72. doi: 10.1038/s41588-017-0001-z. Epub 2017 Nov 27.

Turcan, S., Makarov, V., Taranda, J. *et al.* (2018). 'Mutant-IDH1-dependent chromatin state reprogramming, reversibility, and persistence'. *Nat Genet* 50, pp: 62–72. https://doi.org/10.1038/s41588-017-0001-z

Turkalp Z, Karamchandani J, Das S (October 2014). "IDH mutation in glioma: new insights and promises for the future". *JAMA Neurology*. 71 (10): 1319–25. Doi:10.1001/jamaneurol.2014.1205.

Valente, L. and Nishikura, K., 2007. RNA Binding-independent Dimerization of Adenosine Deaminases Acting on RNA and Dominant Negative Effects of Nonfunctional Subunits on Dimer Functions. *Journal of Biological Chemistry*, 282(22), pp.16054-16061.

Wang AL, Carroll RC, Nawy S. (2014) Down-regulation of the RNA editing enzyme ADAR2 contributes to RGC death in a mouse model of glaucoma. *PloS One*. 7;9(3):e91288. Doi: 10.1371/journal.pone.0091288.

Wang J, Qiu Z, Wu Y. (2018) 'Ubiquitin Regulation: The Histone Modifying Enzyme's Story'. *Cells*. 7(9):118. https://doi.org/10.3390/cells7090118

Wang Y, Xu X, Yu S, Jeong KJ, Zhou Z, Han L, Tsang YH, Li J, Chen H, Mangala LS, Yuan Y, Eterovic AK, Lu Y, Sood AK, Scott KL, Mills GB, & Liang H (2017). Systematic characterization of A-to-I RNA editing hotspots in microRNAs across human cancers. *Genome Research*, 27(7), 1112–1125. 10.1101/gr.219741.116

Wang Y, Zeng Y, Murray JM, & Nishikura K (1995). Genomic organization and chromosomal location of the human dsRNA adenosine deaminase gene: The enzyme for glutamate-activated ion channel RNA editing. *Journal of Molecular Biology*, 254(2), 184–195. 10.1006/jmbi.1995.0610

Wang Y., Chung D-h, Monteleone L.R, Li J., Chiang Y., Toney M.D., Beal P.A., (2019). RNA binding candidates for human ADAR3 from substrates of a gain of function mutant expressed in neuronal cells, *Nucleic Acids Research*, 47(20) Pages 10801–10814, https://doi.org/10.1093/nar/gkz815

Wang, L.; Zhao, Z.; Meyer, M.B.; Saha, S.; Yu, M.; Guo, A.; Wisinski, K.B.; Huang, W.; Cai, W.; Pike, J.W.; et al. (2014) 'CARM1 methylates chromatin remodeling factor BAF155 to enhance tumor progression and metastasis'. *Cancer Cell 25*, 21–36.

Wei, H., Mundade, R., Lange, K. and Lu, T., (2013) 'Protein arginine methylation of non-histone proteins and its role in diseases'. *Cell Cycle*, 13(1), pp.32-41.

Weier, H., George, C., Greulich, K. and Samuel, C., 1995. The Interferon-Inducible, Double-Stranded RNA-Specific Adenosine Deaminase Gene (DSRAD) Maps to Human Chromosome 1q21.1–21.2. *Genomics*, 30(2), pp.372-375.

Wen, A., Sakamoto, K. and Miller, L., 2010. The Role of the Transcription Factor CREB in Immune Function. *The Journal of Immunology*, 185(11), pp.6413-6419.

Williams, N., Ryan, D., Costa, A., Mills, E., Jedrychowski, M., Cloonan, S., Frezza, C. and O'Neill, L., 2022. Signaling metabolite L-2-hydroxyglutarate activates the transcription factor HIF-1α in lipopolysaccharide-activated macrophages. *Journal of Biological Chemistry*, 298(2), p.101501.

Winston F., and Allis CD. (1999). The bromodomain: A chromatin-targeting module? *Nat Struct Biol* 6: 601– 604.

Xenaki, G., Ontikatze, T., Rajendran, R. *et al* (2008). 'PCAF is an HIF-1α cofactor that regulates p53 transcriptional activity in hypoxia'. *Oncogene* 27, pp: 5785–5796 doi: https://doi.org/10.1038/onc.2008.192

Xu X, Zhao J, Xu Z, Peng B, Huang Q, Arnold E, Ding J (August 2004). "Structures of human cytosolic NADP-dependent isocitrate dehydrogenase reveal a novel self-regulatory mechanism of activity". *The Journal of Biological Chemistry*. 279 (32): 33946–57. doi:10.1074/jbc.M404298200.

Yang W, Chendrimada TP, Wang Q, Higuchi M, Seeburg PH, Shiekhattar R, & Nishikura K (2006). Modulation of microRNA processing and expression through RNA editing by ADAR deaminases. *Nature Structural and Molecular Biology*, 13(1), 13–21. 10.1038/nsmb104

Zhang X et al (2005) Genome-wide analysis of cAMP-response element binding protein occupancy, phosphorylation, and target gene activation in human tissues. Proc Natl Acad Sci USA 102(12):4459–4464