Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

REST/NRSF Modulators

Evidence Summary

REST/NRSF confers resiliency to stress and is dysregulated in a variety of age-related diseases; however, its widespread context-dependent effects make it a challenging therapeutic target.

Neuroprotective Benefit: Maintained low-level neuronal REST/NRSF activity can preserve cognition by protecting neurons from aging-associated stressors, and may be a relevant biomarker for neurodegenerative disease.

Aging and related health concerns: Maintained REST/NRSF activity is linked to longevity by enhancing resistance to stress. Dysregulated REST/NRSF is associated with a variety of diseases, including cancer.

Safety: REST/NRSF exerts a multitude of context-dependent functions, so therapeutics toward it will have a high risk for undesired pleiotropic effects. It will need to be targeted in a disease-specific manner. More basic research is needed.

What is it?

Repressor Element-1 Silencing Transcription factor (REST), also called Neuron-Restrictive Silencer Factor (NRSF) is a transcription factor that acts as a regulator of neuron-specific gene expression [2]. It is involved in the process of neurogenesis, where it maintains neural stem cell proliferation, and its downregulation is involved in triggering neuronal differentiation. High levels of REST/NRSF are expressed in non-neuronal cells to induce epigenetic modifications that repress neuronal specific genes. Low levels are maintained in neurons throughout life, where it acts to modify neuron-specific genes in a context-dependent manner. In this way, REST/NRSF is an important regulator of synaptic transmission and excitatory-inhibitory balance in the nervous system.

REST/NRSF interacts with neuron-restrictive silencer element (NRSE or RE1) sequence domains on DNA and interacts with a variety of cofactors to induce epigenetic modifications. REST/NRSF has both N-terminal and C-terminal repressive domains. The N-terminal domain interacts with mSIN3, while the C-terminal domain interacts with CoREST. In combination with mSIN3 or CoREST, REST/NRSF recruits a variety of additional chromatin modifiers, such as histone deacetylases (HDACs) and methyltransferases. REST/NRSF can also serve to activate genes in certain contexts [3]. REST/NRSF has context dependent effects based on the presence of cofactors in a given cell. REST/NRSF is subject to alternative splicing, and the different isoforms are likely to preferentially act with certain cofactors and thus preferentially regulate certain subsets of target genes. REST/NRSF activity is controlled by its cellular localization, as it is active in the nucleus, and inactive when sequestered in the cytoplasm. REST/NRSF is important for the maintenance of neuronal resilience to stress, and its activity is dysregulated in a variety of neurological diseases.

Direct therapeutic targeting of REST/NRSF is challenging because of its pleiotropic, context-dependent effects. It may be necessary to develop drugs to specifically target certain cofactors or complexes in order to limit undesired effects.

Neuroprotective Benefit: Maintained low-level neuronal REST/NRSF activity can preserve cognition by protecting neurons from aging-associated stressors, and may be a relevant biomarker for neurodegenerative disease.

Types of evidence:

- 1 clinical trial assessing effect of mindfulness on REST/NRSF
- 4 gene association studies for REST/NRSF variants and cognitive outcomes
- 4 gene association studies for REST/NRSF variants and brain volume
- 4 studies on REST/NRSF expression in post-mortem brain tissue
- 3 studies on plasma REST/NRSF levels
- Numerous laboratory studies

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function:

Cognitive Aging: REST/NRSF PROMOTES NEURONAL RESILIENCE TO STRESS

REST/NRSF is best understood for its developmental role in neuronal differentiation, however, it has come to be appreciated that REST/NRSF is important for the maintenance of neuronal homeostatic processes throughout the lifespan, such that dysregulation is associated with a variety of brain diseases [1]. REST/NRSF activity is an important component of healthy cognitive aging. Although expressed at low levels in neurons, REST/NRSF levels are elevated in the aging brain in response to stress [4]. The protective response involves the translocation of REST/NRSF from the cytoplasm to the nucleus where it promotes resiliency to stress by repressing genes involved in cell death.

The levels of oxidative and glycoxidative damage increase during the course of aging in the human brain. The breakpoint where oxidative stress homeostasis breaks down and damage to respiration-related proteins accumulates was found to be around 60 years of age [5]. Due to the correlations between REST/NRSF levels and oxidative stress markers, it is hypothesized that these stressors induce REST/NRSF as part of an adaptive response [4; 5]. As such, increased levels of active (nuclear localized) REST/NRSF in neurons in the aged brain is indicative of a healthy (productive) aging response. This induction of REST/NRSF is associated with canonical Wnt/β-catenin signaling [4]. In the Religious Orders Study and Rush Memory and Aging Project cohorts, higher levels of nuclear REST/NRSF in prefrontal cortex neurons were positively correlated with a measure of global cognition, as well as measures of episodic, semantic and working memory [4]. Higher levels of nuclear REST/NSRF were associated with

preservation of cognitive function when features of brain pathology consistent with Alzheimer's disease (AD) were present.

REST/NRSF also plays a role in the regulation of network excitability due to its ability to regulate the expression of various ion channel and neurotransmitter receptor subunits [6]. The repression of genes involved in neuronal excitation may preserve cognitive function by reducing the propensity for excitotoxicity.

Various genetic variants of REST/NRSF have been associated with measures of cognitive aging. Genetic analysis has indicated that REST/NRSF has been subject to strong positive selection pressure over the course of primate evolution, especially in the human lineage [7]. Many of the selected sites occur within a region surrounding the variable number tandem repeat (VNTR), which is the same region in which many of the variants associated with cognitive aging are located. Many of the genes containing RE1 motifs, the site of interaction for REST/NRSF repression, are human-specific and encode genes involved in learning and memory. The variants in REST/NRSF likely influence the specificity toward and/or degree of repression for certain subsets of targets important for cognition. The effects are influenced by haplotype because the activity of REST/NRSF requires a variety of interacting partners. The prevalence of different REST/NRSF variants, and more importantly, (linked) variant combinations, differs within different ethnic populations, thus many of the associations appear to be more apparent in certain populations [8]. As a result, REST/NRSF variants are best understood as risk modifiers with respect to cognitive aging.

The rs3796529 variant of REST/NRSF has been one of the most studied, and the studies indicate that its effects are context-dependent, in that the phenotype is only apparent within certain genetic backgrounds and conditional states. A whole exome sequencing meta-analysis of five cohorts (n=923) (ADNI-1, ADNI-GO/2, IMAS, Add- NeuroMed, and MIRAGE) found that the REST/NRSF functional variant rs3796529 (minor) T allele was associated with larger right hippocampal volumes and greater mean cortical thickness in the right temporal cortex in the context of the ApoE3/E3 genotype [9]. Individuals with mild cognitive impairment (MCI) without the minor allele (C/C genotype) showed a faster rate of gray matter loss over a two-year period. A follow-up analysis including non-Hispanic Caucasians in the ADNI cohort (n=1,566) including all ApoE genotypes found that the effect of the REST/NRSF variant on hippocampal volume was evident in those with MCI or AD, but not in those with normal cognition [10]. An analysis of seven genome-wide association study (GWAS) data sets from the ENIGMA consortium (n= 30,717) failed to find a significant association between the rs3796529 T allele and seven subcortical brain regions (nucleus accumbens, caudate, putamen, pallidum, amygdala, hippocampus, and thalamus) [11]. On its own, the rs3796529 T allele was not significantly associated

with hippocampal volume, but a haplotype containing both the rs3796529 C allele and the rs2227902 T allele was associated with reduced right hippocampal volume in a separate study (n=99, individuals of European descent) [7].

The rs2227902 (minor) T allele is in linkage disequilibrium with a 4-copy VNTR as part of a minor haplotype (9% frequency in a UK population n=746) [12]. This REST/NRSF haplotype was associated with a higher score of general intelligence. It also had an additive interaction with respect to general intelligence with the BDNF Val66 (G) allele. REST/NRSF acts as a repressor of BDNF, and it is thought that this 4-copy variant may have reduced affinity for the BDNF gene, resulting in less repression and higher levels of BDNF. The inverse allele combination (rs2227902 G allele+ 5-copy VNTR with Val66Met A allele) was associated with lower general intelligence. This combination was also associated with epilepsy-related cognitive decline. The REST/NRSF rs2227902 G allele variant was found to be associated with reduced baseline memory function and psychomotor speed, and had an additive interaction with the BDNF rs6265 (Val66Met A allele) variant resulting in accelerated decline following epilepsy diagnosis (n=82) [13].

In a Taiwanese population (n=634), the REST/NRSF rs1277306 A allele was associated with cognitive decline during aging in non-ApoE4 carriers [8]. Interactions were also present, such that those with the rs1713985 TT genotype and rs1277306 A allele had a 2.11-fold (95% Confidence Interval CI 1.18 to 3.76, p=0.0117) increased risk for poor cognitive aging.

Human research to suggest benefits to patients with dementia: None

Mechanisms of action for neuroprotection identified from laboratory and clinical research:

REST/NRSF acts as a transcriptional repressor of neuron-specific genes in non-neuronal cells, thus high expression (and activity) of REST/NRSF in neurons results in the repression of essential neuronal genes [3]. Consequently, REST/NRSF is expressed at a low level in neurons, while its activity is modulated in a dynamic context-dependent manner. The activity of REST/NRSF is highly context dependent because it primarily acts as an epigenetic remodeler, thus its effects are influenced by the chromatin environment and presence of interacting cofactors in a given cell. The activity level is also controlled by cellular localization, such that it is active within the nuclear compartment, and inactive within the cytoplasm. Several neurodegenerative conditions are characterized by levels of nuclear REST/NRSF that are too high or too low. Methods to restore REST/NRSF localization may have therapeutic value. The REST/NRSF locus was identified as one of the most highly enriched differentially methylated regions in the context of aging, characterized by age-related hypermethylation in blood tissue [14]. This age-related repression

of REST/NRSF may contribute to the age-related risk for neurodegenerative disease. Therapeutically, then, the best way to modulate REST/NRSF activity is through the modulation of the cofactors. Moreover, it will be important to select cofactors that confer specificity, since many chromatin remodeling proteins themselves act in a broad manner, such that targeting them would also yield numerous off-target effects.

REST/NRSF is also subject to alternative splicing, and part of the context-dependent effects may stem from differential splicing under different conditions [15]. REST4 is the best characterized isoform. REST4 is neuron-specific and lacks the C-terminal domain, so it cannot work in concert with CoREST to repress target genes. In this way, it may act in a dominant/negative manner to partially antagonize the repressive activity of REST/NRSF. It is only partial because it maintains the ability to repress targets via interaction with mSIN3 through its intact N-terminal domain. Most of the predicted splice variants of REST/NRSF have not been well characterized, but it is anticipated that it may be possible to more selectively target certain disease-relevant aspects of REST/NRSF activity by controlling the composition of isoforms/splice patterns.

Overall, more information is needed about the particular interactions with REST/NRSF that are disrupted or enhanced, as well as the milieu of splice variants involved in each neurological disease, in order to design targeted therapeutic approaches.

Alzheimer's disease: REST/NRSF IS ABSENT FROM THE NUCLEUS IN NEURONS

The increase in nuclear REST/NRSF that promotes resiliency to neuronal stress in the aged brain appears to be absent in vulnerable brain regions, especially the hippocampus and prefrontal cortex, in individuals with AD [4]. Meanwhile, nuclear REST/NRSF remains intact in brain regions that are relatively unaffected in AD. A decline in Wnt/β-catenin signaling in AD may be one of the mechanisms underlying the loss of REST/NRSF induction in AD neurons [4]. In a stage-stratified AD population, the loss of nuclear REST was associated with the epigenetic de-repression of genes associated with AD pathogenesis. REST/NRSF represses a variety of genes implicated in AD pathogenesis, including presenilin 2, p38 MAPK, and several genes involved in tau phosphorylation. The sequestration of REST/NRSF within the cytoplasm due to impaired protein degradation mechanisms may also contribute to the decline in nuclear levels. REST/NRSF was found to be localized with Aβ within LC3+ autophagosomes. Alteration of the nuclear lamina/envelope may also contribute to impaired nuclear import of REST/NRSF [16].

Gene variants in REST/NRSF have been associated with increased or decreased risk for AD, though the particular variant associations tend to differ amongst different ethnic groups, because the effects are dependent on gene interactions and influenced by haplotype. A study using the ADNI cohort (n=1,566 non-Hispanic Caucasians) found that the rs3796529 (minor) T allele was associated with larger hippocampal volumes in AD patients [10]. A separate study using larger data sets (17,008 AD cases/ 37,154 controls and 8,572 AD cases/11,312 controls; European) found a trend toward reduced risk for AD with the rs3796529 T allele (β = –0.0254), but the effect was not significant (p = 0.2101) [17]. This may be a result of genetic heterogeneity and the lack of stratification for ApoE genotype. In Taiwan, the rs3796529 T allele is less common in individuals with AD, and the T/T genotype is associated with protection against cognitive decline (adjusted Hazard Ratio HR:0.593, 95% CI 0.401 to 0.877, p=0.009) [18].

REST/NRSF has been proposed to serve as a biomarker for AD indicative of disease progression and/or to assess treatment efficacy. Plasma levels of REST/NRSF were found to be reduced in AD (M=46.77 pg/mL) relative to healthy elderly controls (M=89.13 pg/mL) (n=62) [19]. REST/NRSF levels were below the limit of detection for many of the AD patients, and better detection could be achieved by assessing REST/NRSF in plasma microvesicles (AD M=110.01 pg/ mL vs Control M=222.43 pg/ mL). REST/NRSF levels declined in relation to severity of impairment (HC>stable MCI> MCI converter>AD). REST/NRSF levels were positively correlated with BDNF levels. In an 8-week intervention RCT for mindfulness training in older adults at high risk for AD (n=81), the training was associated with an increase in REST/NRSF levels relative to placebo. Lower levels of REST/NRSF in plasma neural exosomes were also detected in AD patients relative to controls in a separate study (47.4 ± 8.16 pg/mL vs 667 ± 140 pg/mL) [20].

Parkinson's disease: REST/NRSF IS SEQUESTERED IN CYTOPLASM

In postmortem brain tissue from patients with Parkinson's disease (PD) (n=6), protein levels of REST/NRSF were decreased in dopaminergic neurons of the substantia nigra relative to controls (n=6) [21]. Instead of entering the nucleus, as is found in control aged dopaminergic neurons, REST/NRSF is sequestered within Lewy body aggregates in PD neurons. In mice, REST/NRSF was found to co-localize with p62 and to accumulate within cytoplasmic aggregates, such as Lewy bodies, as a result of autophagy dysfunction. Mice lacking REST/NRSF in dopaminergic neurons are more vulnerable to MPTP-related damage, and have impaired neurogenesis [22; 23]. This suggests that the loss of REST/NRSF activity resulting from cytoplasmic sequestration accelerates neurodegeneration by reducing the capacity of the neurons to withstand oxidative stress.

Frontotemporal dementia: REST/NRSF IS SEQUESTERED IN CYTOPLASM

The levels of nuclear REST/NRSF were found to be reduced in cortical neurons in postmortem brain tissue from frontotemporal dementia (FTD) patients (n=16) [4]. REST/NRSF was instead sequestered in LC3+ autophagosomes along with misfolded proteins. In cases of tau-positive FTD, REST/NRSF was co-localized with phosphorylated tau, and in cases of TDP-43 positive FTD, it was co-localized with TDP-43. In contrast, higher levels of REST/NRSF were detected in plasma neural exosomes in a separate study (2065 ± 162 pg/mL vs 691 ± 123 pg/mL) [20]. It is unclear whether there is a link between the increase of REST/NRSF in autophagosomes and exosomes in FTD.

Dementia with Lewy Bodies: REST/NRSF IS SEQUESTERED IN CYTOPLASM

REST/NRSF was depleted from the nucleus in cortical neurons from postmortem tissue of patients with dementia with Lewy bodies(DLB) (n=18), and was co-localized with alpha synuclein within LC3+ autophagosomes in the cytoplasm [4]. A separate study also found that REST/NRSF protein levels were decreased in the nucleus of neurons in the cortex and substantia nigra [21]. Similarly, REST/NRSF was sequestered in Lewy bodies as part of cytoplasmic misfolded protein aggregates.

Huntington's disease: EXCESSIVE NUCLEAR REST/NRSF IN NEURONS IS HARMFUL

In contrast to other prominent neurodegenerative diseases, nuclear levels of REST/NRSF are elevated in the context of Huntington's disease (HD). While a low level of activated REST/NRSF can be neuroprotective, at high levels, REST/NRSF represses essential neuron-specific genes, leading to loss of cellular function. The Huntingtin protein, HTT, restricts nuclear entry of REST/NRSF as part of a complex that contains RILP, the protein that controls the cellular localization of REST/NRSF. This cytoplasm sequestering activity is lost in mutant HTT (mHTT), leading to nuclear accumulation of REST/NRSF and the loss of neuronal function [24]. One of the affected neuronal genes is the neurotrophic factor BDNF, which is severely reduced in the striatum of those with HD. The restoration of BDNF is one of the therapeutic approaches for HD, and some of these efforts are aimed at relieving REST/NRSF-mediated repression of BDNF.

There have been preclinical studies to identify therapeutic agents to reduce nuclear accumulation of REST/NRSF in HD cells. X5050 was identified in a high-throughput screen as a compound that promotes the degradation of REST/NRSF. When administered intraventricularly in an HD mouse model, X5050 treatment increased BDNF levels in the cortex [25]. C91 is a small molecule designed to interfere with formation of the REST-mSIN3-HDAC repressor complex by targeting mSIN3b [26]. C91 restored BDNF expression in HD cells and mHTT expressing zebrafish embryos. Other studies have found that targeting

the REST-mSIN3 complex can effectively inhibit histone deacetylase (HDAC) activity [27]. An alternatively spliced form of REST/NRSF lacking exon 3, ΔE3, lacks the motif for nuclear targeting. Antisense oligos (ASOs) that enhance levels of ΔE3 reduced nuclear levels of REST/NRSF and restored expression of neuronal genes in HD cells [28]. It is still unclear whether these measures could be safely used without interfering with the essential functions of REST/NRSF in other cell types. It may be necessary to target a (striatal) neuron specific complex.

Epilepsy: REST/NRSF REGULATES NEURAL EXCITABILITY

REST/NRSF is an important regulator of excitatory-inhibitory balance in the CNS [6]. REST/NRSF is a double-edged sword with respect to epilepsy, since its activation may be neuroprotective in the short-term, but lead to detrimental long-term changes to the neural network [2]. The expression of REST/NRSF was found to be increased in temporal cortical tissue from patients with drug-resistant mesial temporal lobe epilepsy (n=28) relative to controls (n=13) (protein levels +273%, $p < 0.01$) [29]. Higher seizure frequency was associated with higher expression. REST/NRSF is overexpressed for around 24 hours in response to seizure activity, and frequent seizure activity may result in chronically elevated REST/NRSF. The acute elevation in REST/NRSF appears to be a protective response to limit hyperexcitability, but its chronic overexpression may promote neural excitability via the alteration of ion channel expression stemming from epigenetic mechanisms. Targeting the REST/NRSF chromatin complexes that pathologically alter ion channel expression may help restore excitatory-inhibitory balance in epileptics. Variants in REST/NRSF may impact epilepsy-associated cognitive decline (see Cognitive Aging section) [13].

Depression: REST/NRSF REPRESSES DEPRESSION/ANXIETY-RELATED GENES

In addition to enhancing resilience to physiological stressors, REST/NRSF is involved in resiliency to psychological/social stress. Several of the target genes of REST/NRSF are implicated in mood disorders, including corticotropin releasing hormone (CRH), BDNF, and the serotonin (5-HT) 1A receptor [30]. In patients with major depressive disorder, mRNA expression of REST/NRSF was found to be reduced, while stress-associated targets such as CRH and pro-inflammatory mediators were increased. REST/NRSF may contribute to the mechanism whereby early life neglect or social stress leads to an increased propensity toward mental disorders later in life and decreased resiliency toward stress, which can result in a shortened lifespan. In rodents, maternal care downregulates glutamatergic transmission on stress-sensitive neurons in the hypothalamus and represses expression of CRH via a mechanism involving REST/NRSF-mediated transcriptional repression [31]. This down-regulation of the stress response protects against anxiety and depressive phenotypes when subject to stress later in life. In contrast,

early-life stress which prevents this process primes the nervous system for a heightened stress-response and adverse outcomes later in life. One of the key components of REST/NRSF-associated resiliency is the repression of pro-apoptotic genes, thus a decline in REST/NRSF activity both reduces the threshold for a stress response and reduces the capacity of neurons to survive in the context of stress. This mechanism may be connected to the association between reduced neural excitation/enhanced REST/NRSF repressive activity and longevity (see Lifespan section) [6].

REST/NRSF targets have been shown to be affected by antidepressants, and the restoration of neuronal resiliency may be a key mechanism in their efficacy [32]. The reversal of the epigenetic marks stemming from REST/NRSF dysregulation may mitigate stress-related mood disorders. The expression and/or activity of REST/NRSF and stress pathway related targets may be useful as biomarkers to assess the likelihood of therapeutic efficacy of antidepressants in a given patient, and to guide the development of more effective antidepressants.

Consideration of REST/NRSF activity will also be important for mitigating the psychiatric symptoms associated with other neurological diseases. In patients with drug-resistant mesial temporal lobe epilepsy, those without mood disorders had higher REST/NRSF expression than those with mood disorders [29]. Psychiatric symptoms are a common feature of advanced stage neurodegenerative diseases where REST/NRSF activity levels are extremely low, such as AD. A clinical trial assessing mindfulness as a treatment for AD found that increased REST/NRSF expression was associated with reduced psychiatric symptoms in older adults at high risk for AD [19].

APOE4 interactions: ApoE4 may modify REST/NRSF-mediated risk in AD. Some gene association studies have found that the impact of some REST/NRSF variants, such as rs3796529, on hippocampal volumes and AD risk are modified by ApoE genotype. The protective effect for the rs3796529 T allele appears to be most prominent in the genetic background of ApoE3, suggesting that the presence of ApoE4 nullifies that protective advantage [9]. The effect may be related to the differential activity of REST/NRSF at a subset of targets, perhaps enhanced repression of pro-apoptotic factors, or increased expression of neurotrophic factors. Whatever the exact mechanism, it likely takes place within the nucleus. A study in AD patient iPSC-derived neurons found that ApoE4 expression was associated with reduced nuclear entry of REST/NRSF [16]. The reduction in nuclear entry may then lessen, or in some cases amplify, the impact of a given REST/NRSF variant. Plasma REST/NRSF levels were also found to be impacted by ApoE genotype [19]. Since the loss of nuclear REST/NRSF is associated with the loss of neuronal resiliency in AD, the presence of ApoE4 may accelerate the loss of neuroprotective REST/NRSF activity.

Aging and related health concerns: Maintained REST/NRSF activity is linked to longevity by enhancing resistance to stress. Dysregulated REST/NRSF is associated with a variety of diseases, including cancer.

Types of evidence:

- 1 study on REST/NRSF activity in postmortem brain tissue for longevity
- Numerous laboratory studies

Lifespan: REST/NRSF MAY REGULATE LONGEVITY PATHWAYS

REST/NRSF expression within the CNS is associated with longevity. There is a distinct transcriptomic signature in the cerebral cortex associated with human longevity that involves the downregulation of genes involved in neural excitation, many of which are regulated (repressed) by REST/NRSF [6]. This may be necessary for the long-term maintenance of neural network homeostasis. REST/NRSF activity levels were found to be increased in the cortical neurons of centenarians relative to individuals who died in their 70s or 80s [6]. The effect was seen at the level of activity rather than expression, such that centenarians showed greater levels of REST/NRSF activity (repressor function) at a given expression level. The connection with longevity may be related to FOXO1. REST/NRSF expression is correlated with FOXO1 expression in the human brain [6]. The levels of activated nuclear levels of these transcription factors are also correlated. The age-related induction of FOXO1 may require the age-related activation of REST/NRSF, as this induction of FOXO1 was absent in mice with REST/NRSF deficient neurons. In an AD mouse model, the intravenous transfer of young blood restored hippocampal levels of REST/NRSF as well as FOXO1 [33].

In *C. elegans*, spr-3/spr-4 (REST/NRSF orthologs)-mediated neural suppression leads to the activation of daf-16 (FOXO ortholog), which is associated with lifespan extension [6]. *C. elegans* containing a C-terminal truncated mutant form of spr-3, spr-3(by108), showed increased resistance to environmental stressors, and their lifespan was extended by 26% [34]. The spr-3(by108) mutant led to increased levels of daf-16. This mutant version resembles the mammalian REST4 splice variant, suggesting that REST4 may be involved in FOXO1 upregulation and mediate the longevity effects of REST/NRSF in humans.

Cancer: REST/NRSF PLAYS DUAL CONTEXT-DEPENDENT ROLES

REST/NRSF has context-dependent effects with respect to cancer. REST/NRSF plays a critical role in the maintenance of neural stem cell populations, and has a corresponding oncogenic role in brain tumors [35]. REST/NRSF is involved in maintaining the self-renewal potential of glioma stem cells. REST/NRSF has been found to be upregulated in medulloblastoma, neuroblastoma, and glioblastoma. The tumorigenic effects involve cross-talk between REST/NRSF and other oncogenic signaling pathways, such

as hedgehog and Wnt signaling. The inhibition of REST/NRSF activity is considered a therapeutic target for these cancers. This has generally involved the use of drugs that reverse epigenetic repressive marks, such as inhibitors of histone deacetylases or methyltransferases [2].

In contrast, REST/NRSF typically has tumor suppressor capacity in non-CNS cancers. Loss of REST/NRSF increases the potential for oncogenic transformation in epithelial cells, and genetic deletion of REST/NRSF is common in colon cancer [35]. Cancer cells often show aberrant expression of neuronal genes due to the expression of alternatively spliced, truncated forms (lacking the repressive domains) of REST/NRSF that can act as dominant/negatives toward the repressive activity of full-length REST/NRSF.

Therefore, it is important to consider the nature of the REST/NRSF dysregulation when developing cancer therapeutics.

Neuropathic pain: REST/NRSF MAY PROMOTE PAIN-RELATED EPIGENENTIC MODIFICATIONS

REST/NRSF is upregulated in the context of neuropathic-pain inducing injuries [2]. The repression of ion channels involved in setting the threshold for pain and analgesic properties by REST/NRSF may play a role in the induction and maintenance of neuropathic pain. Targeting of the cofactors that interact with REST/NRSF to induce these epigenetic modifications may reverse these changes and restore endogenous mechanisms of analgesia.

Cardiovascular: REST/NRSF HELPS MAINTAIN CARDIAC INTEGRITY

REST/NRSF regulates the cardiomyocyte cell cycle, and is important for cardiac development and regeneration in mice [36]. In adulthood, it is important for maintaining cardiac integrity. In mice, the loss of cardiac REST/NRSF leads to the upregulation of fetal cardiac genes, which alters ion channel activity in the heart [37]. This ultimately leads to cardiomyopathy and sudden arrhythmic death. The loss of REST/NRSF-mediated repressive epigenetic marks may play a role in some arrhythmias.

Epigenetic effects mediated by REST/NRSF are implicated in ischemia-related cell death [1]. REST/NRSF is upregulated in the context of cerebral ischemia. While the induction may have an acute effect on enhancing the stability of vulnerable neurons, the associated epigenetic modifications lead to ischemia-induced cell death. Blocking these epigenetic modifications via HDAC inhibitors or REST dominant/negatives is protective in animal models of ischemia. These studies suggest that within the context of ischemia, blocking REST/NRSF-associated chromatin remodeling complexes may have therapeutic value.

Safety: REST/NRSF exerts a multitude of context-dependent functions, so therapeutics toward it will have a high risk for undesired pleiotropic effects. It will need to be targeted in a disease-specific manner. More basic research is needed.

Types of evidence:

- Numerous laboratory studies

There are currently no therapies available that specifically target REST/NRSF, although there are a variety of drugs that indirectly impact REST/NRSF and/or its targets [2]. These include HDAC inhibitors, such as valproic acid, which reverse some of the repressive marks induced by REST/NRSF- containing HDAC chromatin complexes. The difficulty in designing therapeutics toward REST/NRSF is in ensuring specificity for particular targets or cell types of interest due to its widespread expression and context-dependent effects. REST/NRSF cannot be globally inhibited because of its important roles in suppressing neuronal genes in non-neuronal cells and in the maintenance of neural stem cell populations. It also cannot be globally activated because high expression disrupts essential processes in neurons.

For a given condition it will be necessary to determine the root cause of the REST/NRSF dysfunction and/or the specific complexes/interacting cofactors responsible for a pathological effect. Conditions such as epilepsy, neuropathic pain, and depression may require the targeting of particular chromatin remodeling complexes to reverse pathology stemming from epigenetic events. Neurodegenerative diseases may require targeting of autophagy or protein degradation pathways to restore REST/NRSF activity in neurons with cytoplasmic protein aggregates. Enhancement of particular isoforms associated with neuroprotection, such as REST4, may be another strategy. A better understanding of the role of different REST/NRSF splice variants in different disease states will be essential for designing effective REST/NRSF-targeted therapeutics.

Ultimately, the safety profile of REST/NRSF-targeted therapies will depend on their specificity, and due to its context dependency, these therapies will likely need to be tailored to a very specific population to mitigate side effects.

Drug interactions: Potential interactions will depend on the aspects of REST/NRSF activity that are targeted by a particular agent.

Sources and dosing:

REST/NRSF-targeted drugs are not currently available for clinical use. Some drugs that affect chromatin remodelers which interact with REST/NRSF, such as HDACs, histone methyltransferases, and DNA

methyltransferases, are available for preclinical research use, and a few are available for clinical use with a prescription. A variety of antidepressants and antipsychotics also affect REST/NRSF activity.

Non-pharmacological interventions that enhance resiliency toward physiological or psychological stress, including exercise and mindfulness training, have also been shown to boost REST/NRSF activity associated with neuroprotection and resiliency, such as BDNF levels [19; 38].

Research underway:

There are preclinical studies underway toward better understanding the basic biology of REST/NRSF and efforts to design targeted modulators.

Search terms:

Pubmed, Google: REST/NRSF

- Alzheimer's disease, Parkinson's disease, neurodegeneration, aging, lifespan, cardiovascular, cancer, epilepsy, depression, pain

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