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Mini Review

The Contribution of Alternative Splicing to Sex Biases of Aging-Related Phenotypes

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ABSTRACT

Aging-related diseases represent one of the leading public health challenges of our time. While the precise cause of many aging-related diseases remains unknown, proteins termed splicing factors have been specifically implicated in aging-related disorders, including cancer and neurodegenerative disorders such as Alzheimer's disease and amyotrophic lateral sclerosis. Splicing factors regulate the process of alternative splicing, which is a fundamental mechanism of RNA regulation, and thus gene expression. This review will focus on what is known about how inappropriate function of splicing factors and aberrant splicing may contribute to the manifestation of aging-related disease. Importantly, many aging-related diseases also display a sex bias, and this review will explore how alternative splicing may contribute to sex biases seen in aging-related diseases. Evidence that age- and sex-biased RNA processing occurs within the heart, brain and liver, which may contribute to sex and age biases of aging-related diseases within these tissues, will be reviewed. Furthermore, recent studies have found that throughout aging and in the progression of certain age-related diseases there is a distinct sex bias in the expression of splicing factors, as well as, aberrant alternative splicing. While splicing is currently emerging as an important factor in sex-biased aging-related diseases, only a small fraction of known splicing factors have been studied with respect to their sex-specificity and roles in aging. Recent studies underscore the need for alternative splicing profiles to become a standard component of transcriptomic analyses, with sex and age being considered as important biological variables.

KEYWORDS: splicing; alternative splicing; RNA-binding proteins; sex-biased splicing; aging

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ABBREVIATIONS

hnRNP, Heterogeneous nuclear ribonucleoproteins; SR, Serine and arginine-rich; TDP-43 Transactive response DNA-binding protein of 43 kDa

AGINING AND AGING-RELATED DISEASES

Normal aging includes sensory changes such as hearing loss and decreased visual acuity, cognitive aging including mild memory loss, and decline of muscle mass and strength, which can contribute to impaired balance and decreased mobility. Aging-related disorders, on the other hand, include cancer, dementia and neurodegenerative disease, to name a few [1]. It is important to note that a large number of aging-related diseases exhibit a sex bias, such as cancer and Parkinson's disease which are more prevalent in men. In contrast, women are more likely to develop Alzheimer's disease and autoimmune disorders such as multiple sclerosis [2]. Additionally, a sex bias in drug efficacy and adverse reactions exists. Since therapeutic drugs are often developed without regard for such sex biases or without specific consideration of how aging affects drug efficacy, many patients may not receive the appropriate therapeutics or dosages [2,3]. Moreover, a lack of understanding for the molecular basis of sex biases in drug efficacy further prevents appropriate care [3,4].

ALTERNATIVE SPLICING AS A FUNDAMENTAL MECHANISM OF REGULATING GENE EXPRESSION

Changes in alternative splicing patterns are increasingly being observed in senescence and aging [5]. Alternative splicing is an important step in gene regulation and a fundamental mechanism for substantially increasing the complexity of the proteome despite genomes having a comparatively small number of protein coding genes. The process of splicing is carried out by the spliceosome, as well as various splicing factors, including those of the hnRNP and SR families of proteins [6]. Alternative splicing is a process that modifies RNA before it is translated into protein. While general splicing serves to remove intronic sequences from an mRNA, alternative splicing can modify a pre-mRNA transcript in myriad ways. For example, alternative splicing regulates the retention of introns, skipping of exons, or can result in the inclusion of a poison exon, which introduces a premature termination codon, thereby resulting in nonsense-mediated decay of the transcript. The combination of these modifications can create multiple mRNA isoforms, some which impart regulation of the mRNA, such as in the cases of intron retention and poison exon inclusion. Additionally, alternative splicing that regulates the inclusion and exclusion of various exons can result in the production of different protein isoforms from a single gene. Importantly, these different protein isoforms can have distinct properties and therefore different functions in a cell. For a detailed description of the mechanism of splicing, the authors refer readers to [6].

TRANSCRIPTOMIC ANALYSES REVEAL CHANGES IN SPLICING PATTERNS DURING AGING

It is estimated that approximately 95% of all human genes undergo alternative splicing, underscoring the importance of understanding the mechanism by which individual splicing factors regulate their specific RNA targets, and how distinct splice variants for a given gene impart different functions to their unique protein isoform products [5,7]. Transcriptomic analyses have revealed that changes in RNA splicing and RNA processing are strongly correlated with aging [6,7]. Interestingly, the most profound changes in splicing patterns during aging occur within the nervous system [8]. Patients with aging-related diseases including vascular aging, accelerated aging (progeria), Alzheimer's disease, amyotrophic lateral sclerosis, frontotemporal lobar dementia, and myotonic dystrophy have recently been shown to have aberrant alternative splicing patterns. Furthermore, half of the age-related changes in alternative splicing are likely the result of changes in the expression of splicing factors, which cause downstream effects on the splicing of their target RNAs [5,6,9–15]. For example, the expression of seven splicing factors, five hnRNP splicing inhibitors and two splicing activators, in the mouse spleen are associated with lifespan, displaying either increases or decreases in RNA-binding protein expression as a function of age. Of these, HNRNPA2B1 and HNRNPA1, show increased and decreased expression, respectively, and influence parental longevity in both mouse and human populations, suggesting that the regulation of alternative splicing in the aging process is conserved [16,17].

Transcriptomic analyses of blood samples taken from 70-year-old patients over a ten-year time frame, such that all 65 patients were sampled again at age 80, indicated that 294 alternative splicing events are significantly altered with age. Further analysis of these transcripts indicates that many encode proteins that regulate RNA splicing, apoptosis and circadian rhythm. Since disruption of the circadian rhythm is associated with accelerated aging, and apoptosis is associated with neurodegeneration, these results highlight the importance of transcriptomic analyses examining splicing changes, as opposed to only focusing on differential gene expression patterns [18]. Indeed, large scale transcriptomic and splicing analyses identified 49,869 age-associated splicing events across 48 tissues from 544 human subjects. These age-dependent changes in splicing patterns include both an upregulation and downregulation of specific splicing events. Interestingly, most of these events are tissue specific, such that each tissue has a unique age-dependent splicing profile. Furthermore, this comprehensive study concluded that genome-wide splicing profiles are a better predictor of biological age than differential gene expression profiles [5]. Microglia have also increasingly been implicated in aging and aging-related disorders. The generation of a Microglia Genomic Atlas, which compared glial transcriptomic signatures across human aging and aging-related diseases,

uncovered aberrant splicing events that may contribute to the manifestation of aging-related disorders such as Parkinson's disease, Alzheimer's disease and multiple sclerosis, each of which display a sex bias in incidence [19].

INTRON RETENTION IS PREVALENT IN ALZHEIMER'S DISEASE

Intron retention is a process regulated by alternative splicing that results in specific introns being retained within the mature RNA. An increase in intron retention has been observed across aging in both *Drosophila* and mice [20]. Recent studies have identified that there is an increase in intron retention in humans across aging, as well as in Alzheimer's patients compared to age-matched control patients. In the cerebellum alone, there are an estimated 3800 instances of intron retention in Alzheimer's patients that are not observed in control patients. Transcripts that exhibit intron retention are important for the regulation of mRNA stability, transcript localization, and protein homeostasis. Thus, increased intron retention in these transcripts may play a role in aging and Alzheimer's progression [21]. Increases in intron retention have also been observed in a sex-specific manner. In *Drosophila,* over 800 instances of sex-specific intron retention have been observed. In many instances of intron retention, there is also an alteration of gene expression [5]. Nonetheless, it is not understood how sex-specific intronic retention could influence aging and aging-related disease progression.

SEX-BIASED SPLICING OF AUTOSOMAL GENES

Sex-biased splicing has also been observed in many different tissues including the brain, muscle and liver. In a study using postmortem brain samples from 137 individuals, 448 genes were found to have sex-biased expression. Additionally, 85% of these 448 genes displayed sex-biased alternative splicing patterns [22]. Interestingly, 95% of genes that exhibit sex-biased splicing are found on autosomes, refuting a simple link to a sex bias originating in X chromosome dosage [4]. Importantly, sex-biased gene expression, and particularly sex-biased splicing, may contribute to sex differences in disease [2,6]. Indeed, the transcriptomic analysis of postmortem brain tissue showed that 114 genes that display sex-biased expression are also associated with human disease as indicated by their entry into the Online Mendelian Inheritance in Man (OMIM) database. Nonetheless, only 12 of these genes map to a sex chromosome, whereas 39 of these genes demonstrate sex-biased splicing within diseases that manifest in a sex-specific manner. Furthermore, immune related pathways are significantly represented in sex-biased gene expression in postmortem brain tissue of females. This may not be surprising since females are more susceptible to autoimmune disorders such as multiple sclerosis [22]. Interestingly, the sex-biased splicing of immune related genes may be conserved among primates [23].

TISSUE SPECIFIC EXPRESSION OF ALTERNATIVE SPLICING FACTORS

Despite the fundamental importance of splicing to aging-related diseases, relatively few splicing factors have been studied in detail, especially with respect to their sex-specific and aging functions. Surprisingly, only 2%–6% of RNA-binding proteins show tissue-specific expression in humans. Yet, the nervous system shows higher levels of splicing and other forms of RNA regulation than other tissues. Thus, although most RNA-binding proteins do not have tissue-specific expression, dysfunction of ubiquitously expressed RNA-binding proteins often results in tissue-specific pathologies, which often more severely affect the nervous system as compared to other tissues [10,24]. Not surprisingly, RNA-binding protein dysfunction is implicated in many neurodegenerative diseases such as amyotrophic lateral sclerosis, frontotemporal dementia and Fragile X-associated tremor/ataxia syndrome [25]. While tissue-specific expression of RNA-binding proteins is rare, the levels of RNA-binding protein expression may direct tissue-specific RNA processing. Indeed, evidence suggests that certain RNA-binding proteins are differentially expressed in a sex- and age-specific manner. For example, in examining the expression of 1344 RNA-binding proteins in the human liver, it was found that the level of expression of 88 RNA-binding proteins is significantly associated with age, whereas 45 RNA-binding proteins are expressed in a sex-specific manner within the liver. Furthermore, a higher proportion of RNA-binding proteins are expressed in an age-dependent manner than non-RNA-binding proteins, suggesting that RNA-binding proteins play a significant role in aging of certain tissues. Importantly, the RNA-binding proteins that are upregulated in an age-dependent manner in human liver tissue show similar trends in the mouse liver, suggesting that the age-dependent expression of RNA-binding proteins is conserved [26]. Tissue specific functions for splicing factors have only recently been explored. Nonetheless, evidence suggests that tissue-specific knockdown of certain ubiquitously expressed RNA-binding proteins results in distinct phenotypes in *Drosophila.* For example, dysfunction of the RNA-binding protein Caper results in decreased longevity, aberrant motoneuron morphology, as well deficits in adult locomotion that are exacerbated with age and display a sex bias. Tissue-specific knockdown of *caper* within neurons or glia recapitulates the deficits in locomotion that worsen across aging. However, knockdown of *caper* within muscle does not recapitulate certain defects in motoneuron morphology and results in mild adult locomotor deficits that are not exacerbated with age. Taken together, these results reveal distinct roles for *caper* within the nervous system, as compared to muscle [27].

While few RNA-binding proteins have been investigated in the context of aging, evidence suggests that the expression of specific RNA-binding protein isoforms have unique functional consequences. The RNA-binding protein hnRNP DL has two isoforms, a short S-DL and a long L-DL isoform.

The L-DL isoform is preferentially expressed in neurons, where it regulates the splicing of cytoskeletal and synaptic proteins. Downregulation of L-DL is associated with cognitive dysfunction, while overexpression of L-DL in murine Alzheimer's disease models improves cognition and restores splicing of synaptic mRNAs, linking L-DL function to aging and aging-related diseases [28]. Furthermore, modulating the expression of L-DL may provide a new avenue for developing therapeutics for Alzheimer's patients.

ABERRANT ALTERNATIVE SPLICING IN STROKE AND EPILEPSY

One example of a neurological disease that shows a distinct sex bias and changes throughout aging is stroke. Among young people, stroke affects men more than women, however, with increasing age, women are at a much higher risk of stroke than men, making the overall risk of stroke higher in women [29]. 70 genes have been identified as being differentially spliced in blood samples of stroke patients compared to non-stroke patients. Of these, 24 genes are differentially spliced in men and only two in women, suggesting that aberrant splicing may underly an increase in stroke risk [30]. Nonetheless, it remains unknown how differential splicing of these genes influences stroke risk changes across ages.

Epilepsy is a neurological disorder characterized by seizures that displays both a sex and age bias, with men, infants and elderly patients being disproportionately affected [31]. Aberrant splicing and the dysfunction of splicing factors has been associated with epilepsy, and therapeutics that specifically correct splicing defects in epilepsy patients show promise [32–38]. However, it is also evident that alternative splicing can affect therapeutic efficacy. For example, Glucocorticoid Receptors (GR) function in blood-brain barrier efflux and have an α and a β splice form, with α being the common one. It has recently been shown that an increase of the GRβ spliceform may play a role in pharmacoresistance in epilepsy [39]. An aberrant GRα/GRβ ratio in the endothelial cells of epilepsy patients was found to be both age- and sex-dependent, with an aberrant ratio detected in patients over 45 years of age. Furthermore, females specifically exhibited a decrease in the GRα/GRβ ratio that was not seen in male patients [39]. Taken together, alternative splicing of GR in favor of the β isoform represents an aging phenotype in epilepsy that also shows a sex bias that may influence the efficacy of established therapeutics.

ALTERNATIVE SPLICING DEFECTS IN ALZHEIMER'S DISEASE

It has become evident that there is a strong correlation between epilepsy and Alzheimer's disease, both of which are associated with aging [40]. Furthermore, aberrant alternative splicing of a large number of genes has been observed in patients with Alzheimer's disease [41]. For example, *TREM2*, a well-established Alzheimer's disease risk gene, is a myeloid membrane protein implicated in late onset Alzheimer's. Importantly, aberrant splicing of the *TREM2* mRNA confers a greater susceptibility to

Alzheimer's disease. Transcriptomic analysis of human brains from patients with Alzheimer's disease showed that some low-frequency genetic variants of *TREM2* are prone to being alternatively spliced to exclude exon 2, while the overall expression is retained [42]. Exon 2 skipping leads to structural changes that impair the active site of the protein, and therefore its function. Interestingly, TREM2 interacts with Clusterin (CLU), whose dysfunction is also implicated in Alzheimer's disease and may be a factor that contributes to a greater Alzheimer's disease susceptibility in females [43,44]. *CLU* is alternatively spliced by intron inclusion within exon 5, which generates either an extracellular or intracellular protein isoform. In patients with an Alzheimer's disease risk variant rs7982 of the *CLU* gene, there is a significant sex-dependent association of intron retention, with female Alzheimer's patients having a decrease of intron retention compared to healthy adults [44]. Thus, intron retention may play a role in the sex-biased incidence of certain neurodegenerative disorders.

THE ROLE OF THE SPLICING FACTOR TDP-43 IN NEURODEGENERATIVE DISEASE

The Transactive response DNA-binding protein of 43 kDa, or TDP-43, is an alternative splicing factor that has been implicated in several neurological diseases including frontotemporal dementia, amyotrophic lateral sclerosis, and motor neuron disease. Importantly, the risk of amyotrophic lateral sclerosis increases with age, and shows a substantial sex bias, with males being disproportionately affected [45]. Furthermore, sex is an important factor in the clinical manifestation of frontotemporal dementia phenotypes [46]. Within these diseases, cytoplasmic inclusions are found in neuronal cells that are ubiquitin-positive, tau-negative, and positive for TDP-43 [46]. TDP-43 has also been specifically implicated in aging by facilitating changes in gene expression by repressing the splicing of a series of non-conserved cryptic exons [47]. The inclusion of these non-conserved exons within nervous tissue from amyotrophic lateral sclerosis patients is positively correlated with age [48]. In murine amyotrophic lateral sclerosis models, loss of splicing facilitated by TDP-43, correlates with an increase in the senescence marker p16, which contributes greatly to the process of aging [49]. TDP-43 is also associated with other aging-related diseases that display a sex bias such as Alzheimer's disease, where TDP-43 cytoplasmic inclusions are detected in 25% to 50% of patients and especially those with more severe phenotypes and pathology. Importantly, such TDP-43 inclusions are not detected in normal elderly patients, but it is not clear if TDP-43 inclusions are causative of disease or instead a consequence of disease manifestation [50].

Aggregates such as the tau-negative neuronal cytoplasmic inclusions found in many age-related diseases can contain not only TDP-43 but many other proteins that regulate alternative splicing. The sequestration of these proteins inside aggregates or stress granules can lead to changes in gene

expression by sequestering splicing factors from their target RNAs so they can no longer carry out their functions [51]. Furthermore, the formation of cytoplasmic aggregates causes toxicity which may also affect gene expression [52]. It is possible that changes in gene expression that result from TDP-43 cytoplasmic aggregates could contribute to disease progression, as well as aging.

SEX-BIASED GENE EXPRESSION IN NON-NEURONAL TISSUE

While we have focused on the role of alternative splicing in sex-biased neurological aging-related disorders, many other tissues display sex-biased gene expression and disease. For example, heart disease is another age-associated disease that shows a sex bias. A recent transcriptomic and proteomic analysis in the murine heart during aging attempted to elucidate the effects of sex and age on alternative splicing in the heart [53]. Differential exon usage was found for 2315 genes depending on age and 1056 genes depending on sex. When alternative exon usage was analyzed, 1168 genes showed an age by sex interaction. Furthermore, these changes in splicing were found in functionally coordinated genes associated with RNA metabolism, ribosome, nonsense-mediated decay and mitochondrial metabolism. This suggests that RNA processing is heavily implicated in cardiac aging and may help explain sex and age biases in the risk of heart disease. Interestingly, general gene expression differences do not overlap with differential exon usage. Yet again, this suggests that alternative splicing may influence a sex bias in aging independently from general differential gene expression [44].

CONCLUSIONS

In conclusion, alternative splicing is increasingly emerging as a significant factor in the process of aging, and aberrant splicing is increasingly being associated with aging-related disease. While transcriptomic analyses have mainly evaluated differential levels of gene expression in the past, it is evident that going forward, an additional focus on alternative splicing must become standard when examining gene expression. This is underscored by recent studies demonstrating that alternative splicing profiles are distinct from profiles of differential levels of gene expression. Furthermore, due to the prevalence of sex biases in disease, as well as sex-specific profiles of the levels of gene expression and sex-specific profiles of alternative splicing, sex must become a standard variable in gene expression studies. While much is still unknown about the mechanism in which these sex-specific alternative splicing events regulate aging and age-related disease progression, an increasing number of sex-specific alternative splicing events are linked to aging and age-related diseases such as epilepsy, heart disease, amyotrophic lateral sclerosis, and Alzheimer's disease. Many of these observed changes to alternative splicing patterns within aging or disease states require further analysis into the downstream effects of how these changes influence the

aging process. In particular, few studies have investigated whether alternative spliceforms generate functionally distinct protein products. However, such studies are paramount to providing the most effective therapeutics that account for the age of patients as well as differences across sexes.

DATA AVAILABILITY

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

AUTHOR CONTRIBUTIONS

BRF, CM and ECO wrote the paper with input from all authors.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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