Age-Related Macular Degeneration and RNA Research

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Abstract

The scientific understanding of classifications of RNA and the search for a cause and cure for Age-Related Macular Degeneration has changed dramatically in the past 10 years. Comparing two research studies almost a decade a part, but with similar topics, demonstrates how the science has become much more precise. To fully appreciate how quickly researchers are mining the RNA information, one must understand the increasingly rapid expansion of technology. Not only are the laboratory tools used to see the molecular structures of our bodies advancing, but so are the bioinformatic tools such as free on-line global databases with accompanying computer programs to analyze the information. Now more than ever, scientists are able to see the latest research, make a hypothesis, run the idea through computer aided models, and then test it in the lab.

Keywords: Age-Related Macular Degeneration, ncRNA, lncRNA, circRNA, bioinformatics, aging

Age Related Macular Degeneration and RNA Research

Overview of age-related macular degeneration

The third cause of worldwide blindness is Age-Related Macular Degeneration (AMD), but AMD is the main cause of blindness in more developed nations (Berber et al., 2017). There are two types of AMD. Choroidal neovascularization (blood leaking into the eye) also known as Wet AMD has a possible treatment. Dry or atrophic AMD which is more common has yellow-rich deposits known as drusen that collect in and around the macula (Bhattacharjee et al., 2016). These deposits lead to geographic atrophy of the retinal pigment epithelial (RPE) cells which are vital to keeping the macula in a balanced state (Chen et al., 2015). Drusen accumulation is not the only cause of AMD. When mechanisms such as microRNA (miRNA) that maintain the stable expression of genes and proteins are mutated or over-produce or under-produce, the imbalanced cells also become factors of AMD (Berber et al., 2016).

Comparison of Similar Research from 2011 and 2020

Ten years ago

As expected, the research from 10 years ago laid the foundation for today's research through the purification of subsequent research. Friedrich et al. (2011) wrote in their article *Risk-and Non-risk-associated variants at the 10q26 AMD locus influence ARMS2 mRNA expression but exclude pathogenic effects due to protein deficiency* about expression of two genes HTRA1 and ARMS2. HTRA1 is important in making proteins on Chromosome 10q26. ARMS2 is also located on the 10q26 area of Chromosome 10 and known to be related to AMD. The study of these two genes was done by using what was available at the time. Friedrich et al. (2011) discusses using the best-guess genotypes and using the CEU HapMap data release #22 from the International HapMap Consortium 2007. The best-guess genotype was a statistical method to understand unknown genotypes and the HapMap Consortium 2007 was a popular genotype reference database, but Friedrich et al. (2011) determines these tools were insufficient to fully distinguish the actions of the 15 known variants found on 10q26 and to understand the complexity of RNA. Their conclusion was that "our results suggest that currently unknown mechanisms mediate the pathogenic effect of the risk-associated variants at the 10q26 risk AMD locus" (Friedrich et al., 2011).

Current Research

In 2021, Williams et al. (2021) published an article entitled *Chromosome 10q26–driven age-related macular degeneration is associated with reduced levels of HTRA1 in human retinal pigment epithelium*. This investigation covers the same chromosome 10q26, HTRA1 gene, and risk/non-risk

alleles as the Friedrich et al. (2011) paper, but the conclusions were very different because of the advancements of sample collection, data analysis, and technology. The first major difference is the samples. The samples came from the world's largest repository for eye tissue in the world. This enabled the research team to select specific, multi-variable eye tissue for study. One variable is age of donor. Because AMD is a disease that progresses with age, accurate sample comparisons require the samples be of similar age. Friedrich et al. (2011) had a large selection of retinal tissue for the time, the collection, storage, and categorization of the tissue cannot compare to what is available today. Because of the specificity of the tissue samples, William et al. (2021) analyzed the data with greater accuracy. Williams et al. (2021) used more advanced equipment, methods and protocols for working with genomes than were available to Friedrich et al. (2011).

One more important difference between the two studies is the understanding of the Retinal Pigment Epithelium-Bruch's membrane (RPE-BM). The RPE is a membrane that is the barrier and a regulator for the macula. It protects the photoreceptor area by allowing nourishment in and allowing refuse out (Boulton & Dayhaw-Barker, 2001). The BM is a membrane between the RPE and the choroid that regulates the blood flow from the choroid to the RPE (Pro Visu, n.d.). The boundary between the RPE-BM is where most of the irregularities of AMD begin. Williams et al. (2021) noted that Amyloid-Beta drusen is mainly located on the RPE side of the RPE-BM which indicates the drusen is not carried through the RPE-BM but instead is collecting on the RPE side and spreading over the macula resulting in vison loss. After proving that the AMD samples had lower levels of the Htra1 protein (Htra1 protein/HTRA1 gene) compared to the non-AMD samples, Williams et al. (2021) concluded that Htra1 helps maintain a healthy RPE as a person becomes older. In contrast, Friedrich et al. (2011) discusses how the AMD risk alleles have little effect on HTRA1 promoter activity in the retina concluding that unknown mechanisms were causing the problems. Because of the advancement of technology, Williams et al. (2021) believes Htra1 augmentation may be a possible therapy for AMD and is seeking to work with non-human primates to continue the investigation.

RNA Research

Various Forms of RNA

It has long been accepted and taught that there are three main types of RNA whose function is protein formation. Messenger RNA (mRNA) which holds the information from the DNA works with

Transfer RNA (tRNA) to form the polypeptide chains that Ribonsomal RNA (rRNA) use to make the proteins (Lakna, 2017). Scientists have long known about non-coding DNA (ncDNA) and non-coding RNA (ncRNA) which are smaller bits of a polypeptide chain, but considered them "junk DNA" because they did not create proteins and because the ncRNA was thought to be left-over from gene splicing. Working with other non-coding RNAs and proteins, ncRNA form a network of interactions regulating all cellular processes (Wawrzyniak et al., 2018). Using the latest technology, scientists have classified an ever-growing number of ncRNA as vital to cell function including: gene regulation, transcription, DNA replication, response to DNA damage and repair, splicing, turnover, translation and signaling pathways (Statello et al., 2021).

One of the main mechanisms that ncRNA use is competitive binding. The mRNA or sense strand needs to bind with miRNA that match its sequence to form a protein or gene. In competitive binding, ncRNA and miRNA vie to match with the mRNA sequence. When the ncRNA binds to the mRNA, the ncRNA essentially blocks the miRNA from binding which prevents protein formation (Zhang et al., 2020). The ncRNA that blocks the formation of a protein comes from the antisense strand or complementary strand to the sense strand and is called the antisense ncRNA. This important mechanism for preventing or silencing the formation of a protein or gene is called antisense transcription (making of the protein or gene) and may be a means of cell self-regulation (Pelechano & Steinmetz, 2013). Understanding how the delicate balance of sense and antisense mechanisms maintain the homeostasis of a cell is one of the keys to understanding the causes of AMD.

Two classes of stable non-coding RNA are providing new information into possible biomarkers and therapies for AMD (Dhamija & Menon, 2018). Long non-coding RNA (lncRNA) and circular RNA (circRNA) are imperative for proper gene functions. By using the mechanism of competitive binding, lncRNA and circRNA can gather a specific miRNA in large quantities becoming a sponge or reservoir of that particular miRNA. This action slows the protein or gene building of the mRNA. Also, these sponges or reservoirs have been seen to transport the miRNA to another location. This sponge/reservoir mechanism is potentially a natural way to bring homeostasis to the cell by either ridding the area of the unwanted miRNA or by transporting the miRNA to a place where it is needed (Zhang et al., 2020). Upregulation (more than normal) or downregulation (less than normal) of a particular ncRNA may be an indicator of disease. When an ncRNA is either more or less than normal, the scientists look for the companion sense or antisense ncRNA to determine if the normal sense/antisense relationship is out of balance or if the ncRNA in question is exceedingly lacking or over-abundant. Researchers have been

experimenting with using the sense/antisense ncRNA to turn on the production or silence the overabundant production of a protein in hopes of slowing the progression of AMD. (Wawrzyniak et al., 2018).

LncRNA

As their name suggests, long non-coding RNAs are >200-nucleotide-long RNA molecules. Although lncRNA do not generally code, studies indicate they may code in specific circumstances. LncRNA regulate the formation of proteins and genes through various means such as: direct transcriptional regulation, interaction with DNA, RNA, and proteins, and gene regulation (Wawrzyniak et al., 2018). When lncRNA are not working properly, cell function is disrupted often causing disease. Finding the dysregulated lncRNA in AMD patients may provide biomarkers for early detection and therapies. For example, the Vax2 gene is a regulator of eye development in mice. Vax2os1 and Vax2os2 are retinal specific, antisense transcript (silencing companion) lncRNA of Vax2. Mice with wet AMD characteristics have downregulated Vax2os1 and Vax20s2 which makes both lncRNA potential biomarkers for wet AMD in mice. Research has discovered 64 lncRNA as possible biomarkers for dry AMD in humans. After mapping the dysregulated (either up or down regulated) lncRNA and mRNA, the dysregulated lncRNA and mRNA were found mostly in the phototransduction and purine pathways (Wawrzyniak et al., 2018). As shown in the research by Williams et al. (2021), knowing the location of the disruptions in a normal system gives the research team a place to investigate. Then, the scientist can determine why there is a problem at that location.

CircRNA

The even newer circular RNA (circRNA) are regulatory RNA molecules which have closed loop structures with no 5'-cap (top end of linear RNA) and no 3'-polyandeylated tail (the bottom end of RNA) (Su et al., 2021). CircRNA are stable partially because of being circular. Linear RNA can be destabilized by ribonuclease degradation, but circRNA do not have terminals (end of chain) for binding therefore ribonuclease degradation generally does not happen. A chain of new investigations about CircRNA have rapidly improved our understanding of what it is and does with a focus on binding sites and splitting. Currently, circRNA is classified into 3 component-based subtypes: exonic circRNA found in the cytoplasm, intronic circRNA found in the nucleus, and exon-intron circRNA found in the nucleus. CircRNA are often thought of as regulators. Two examples of this are the previously discussed ability of circRNA to act as a transport sponge/reservoir to move miRNA to a different area in the cell or to repress miRNA

expression. CircRNA have been known to regulate alternative splicing and to support protein or peptide translation (Wawrzyniak et al., 2018). Recent AMD research indicates that because circRNA can be found in human saliva and blood, specific circRNA can be non-invasively gathered biomarkers for stressed RPE cells. The study indicates that circNR3C1 regulate specific miRNA on chromosome ten, but in people with AMD, circNR3C1 was downregulated. These samples were blood serum samples taken from living people with AMD, not post-mortem retinas, meaning that a simple test may be able to target a specific cause and provide a therapy for dry AMD. (Zhang et al., 2020)

Bioanalytical Tools and Data Bases

Statistical research is an important tool for understanding the possibilities of laboratory research. The depth of the databases and the power of new analytical tools have greatly expanded the accuracy of the statistical research. With the National Institute of Health's free of charge databases, anyone in the world can comb through the data to test a hypothesis. This allows a research team to have huge sample sizes that require specialized computer programs such as the Database for Annotation, Visualization and Integrated Discovery (DAVID) for in-depth analytical reports generated very quickly. The DAVID website claims DAVID can "efficiently upload and analyze a list consisting of <=3000 genes. All DAVID tools have been tested with lists in this range and should return results in a few seconds to no more than a few minutes" (LHRI, 2020). Research is no longer bogged down by humans counting, categorizing, and repetitively calculating mountains of data. A hypothesis can be analyzed, the variables changed and analyzed again with multiple tries in just a few hours.

One use of these databases is to find the co-expression network of a particular RNA. In order to determine the role of a lncRNA, Zhu et al. (2017) searched the Gene Expression Omnibus (GEO) Repository at the National Center for Biotechnical Information from the National Institute of Health in the USA to find lncRNA/mRNA pairs. Because Zhu et al. (2017) knew the function of the mRNA, the function of the lncRNA could be ascertained. This process allows a researcher to eliminate unlikely test samples thus creating higher probability of finding the specific samples needed to test a hypothesis. Another use of bioinformatics is to create a construct. For example, Su et al. (2021) used the Gene Expression Omnibus, Starbase database analysis, and RNA hybrid assays to create/simulate a circRNA-miRNA-mRNA network. They were able to test theories on this simulated network to discover a specific node that may be where inflammation and angiogenesis (growth of new blood vessels which is a defining characteristic in wet AMD) occurs causing AMD. Simulated results must be tested again in real life, but the variables, parameters and specific targets have a much higher probability of success

meaning more laboratory time is spent on the actual problem and less time spent eliminating non-factors.

Conclusion

The power of the new field of bioinformatics to analyze the ever-growing data base in AMD research gives scientists the ability to test hypotheses with multiple variables almost instantaneously. They have the ability to reconfigure the variables for analysis of multiple sets in very little time. This new information has deepened the scientific process. The fast, on-line publishing of results allows for quick turnaround time for new ideas to be tested making a tsunami of new theories and results. Coupled with the technological advancement of the hardware in nanobiology, what was impossible to know 10 years ago, is now rapidly being discovered. The understanding of how the mRNA subsets of lncRNA and circRNA work is being studied to find biomarkers and therapies for the causes of AMD giving hope to those who have always been told there is no cure.

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