Chapter 1

Food Allergy: Molecular Basis and the Potential Novel Role of microRNAs

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Abstract

Food allergy is a growing public health problem worldwide, with surveys suggesting that between 2-10% of people are affected at some level. However, the causes, symptoms and severity of food allergies vary widely among individuals. Moreover, although many of the immunological mechanisms underlying food allergy have been investigated, the reasons why some consumers develop allergies but others do not are still not fully understood.

In this chapter, we give an overview of current knowledge concerning the epidemiology and immunology of food allergies. We also discuss recent developments in the diagnostic and intervention strategies available to allergy patients, along with their limitations. Furthermore, we review recent discoveries in the biology of microRNAs – small, non-coding RNA molecules that are ubiquitous regulators of gene expression in eukaryotes – and how they may influence allergic responses. In particular, we discuss the intriguing possibility that extracellular microRNAs present in the circulation or absorbed from ingested foodstuffs could be used to develop novel treatments for food allergy.

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1. The Modern Food Allergy Epidemic

Food allergies are caused by an inappropriate immunological response to certain dietary proteins. When a person with a food allergy consumes or comes into contact with allergenic food, their immune system incorrectly recognizes the food protein as harmful and initiates an immunological reaction. This reaction results in the secretion of allergen-specific antibodies, typically immunoglobulin E (IgE), which cause the release of substances such as histamine, resulting in a variety of allergy symptoms. Severity of food allergies can range from mild to severe, and might affect different parts of the body, including the skin, gastrointestinal tract, respiratory system, and cardiovascular system (Waserman et al., 2018). Common symptoms of a food allergy may include fatigue, swelling, shortness of breath, urticaria, gastrointestinal distress, vomiting, muscle pain and in severe cases, anaphylaxis, a potentially fatal reaction requiring immediate medical intervention (Żukiewicz-Sobczak et al., 2013).

Although specific foods that cause allergies vary from person to person, according to a recent study of Health Canada, cow's milk, egg, peanut, tree nuts, fish/shellfish, wheat, sesame seed, soya and mustard are among the most common allergenic foods. Individuals with food allergies need to carefully manage their diet and be aware of potential allergens in order to avoid allergic responses (Waserman et al., 2018).

1.1. Prevalence

The incidence of food allergies varies according to the population investigated and the allergens under consideration. Indeed, determining food allergy prevalence with certainty is incredibly difficult, due both to the huge variety of possible allergens and symptoms and methodological limitations. However, research has revealed that food allergies are becoming more widespread, particularly among children. The exact causes of the rising prevalence of food allergies are not fully understood. However, various explanations have been proposed to explain this pattern such as the hygiene hypothesis, changes in dietary habits, environmental and epigenetic factors (Prescott & Allen, 2011; also see 'Risk Factors' below).

In general, it is estimated that around 2-10% of the population worldwide is affected by food allergies (Loh & Tang, 2018). The prevalence of food allergies can vary significantly between different countries and regions. For example, Western countries, such as the United States and the United Kingdom, tend to have higher rates of food allergies compared to developing

nations. However, recent research suggests that prevalence is also increasing in developing countries (Loh & Tang, 2018).

While many children outgrow their food allergies, some persist into adulthood. For example, allergies to nuts, fish, and shellfish are more likely to remain throughout life. In contrast, milk, egg, soya, and wheat allergies are often outgrown (Sicherer et al., 2020).

1.2. Risk Factors

Several risk factors have been identified for the development of food allergies. While having one or more risk factors can increase one's chances of acquiring a food allergy, many individuals with the same risk factors never develop one. The interplay between genetics, environmental factors, and immune responses is complex and still not fully understood.

Allergies have a genetic component, and people who have allergic family members are at greater risk (Gerrard et al., 1976; Misiak et al., 2009). A family history of allergies, especially among direct family members such as parents or siblings, increases the likelihood of developing a food allergy. A survey of 622 adults and children reported that the likelihood of a child developing peanut allergy is increased sevenfold if he or she has a parent or sibling who is allergic to peanuts (Hourihane et al., 1996). Similarly, individuals who have a personal or family history of allergic disorders such as asthma, eczema, or allergic rhinitis are more likely to develop food allergies. This suggests a shared underlying allergic tendency (Turnbull et al., 2014).

The "hygiene hypothesis" was first described in relation to asthma (Strachan, 1989) and suggests that reduced exposure to infectious agents and certain microorganisms in early childhood, as a result of improved sanitation and smaller family size, may impact immune system development and so contribute to allergic sensitization. The same concept has been proposed in food allergy (Lack, 2008), where it may also be related to changes in the gut microbiota. Increased antibiotic use and consumption of processed foods are thought to reduce gut microbiome diversity, with possible effects on immune development.

Clinical researchers have tried to correlate the occurrence of food allergy with the timing of introduction of potentially allergenic foods into the infant diet, the duration of breastfeeding, and presence of allergens in the diet of breastfeeding mothers; however, different studies give conflicting results (Järvinen et al, 2019). Studies in animal models suggest that cytokines and immune complexes present in breast milk help to induce tolerance to potential food allergens, suggesting that their early introduction alongside

breast milk may be beneficial. However, at least one study found that longer periods of breastfeeding correlated with an increased incidence of atopic allergies (Bergmann, 2002).

Environmental factors may play a role in the development of food allergies. Repeated exposure to airborne allergens such as pollen, dust mites and pollution are thought to increase the risk of sensitization and food allergy development. Certain other factors like being born via Caesarean section, lack of vitamin D, being of certain ethnic backgrounds, and even certain seasons of birth have been correlated with an increased risk of food allergies in some studies (Lack, 2008; Matsui et al., 2019). However, further research is needed to determine whether these factors have a causal role.

2. How Does Food Allergy Develop?

2.1. Molecular Onset of Food Allergy

The molecular processes underlying food allergy involve a complex interplay between the immune system, specific allergenic proteins, and the individual's genetic predisposition. In fact, food allergies are a group of clinico-pathological conditions, not a single disease (Anvari et al., 2018). Some examples of allergenic dietary proteins include casein in milk, ovalbumin in eggs and cupins in peanuts (Mueller et al., 2014; Dantas et al., 2017 & Docena et al., 1996). These proteins have little similarity with each other but all share general characteristics that enable them to interact with the immune system and potentially trigger an allergic response. They are resistant to degradation by heat, enzymes and low-pH conditions, which makes them stable throughout the gastrointestinal tract (Moriyama, 2015). They are also among the most abundant proteins in their respective foodstuffs, meaning that immune cells will more frequently come into contact with intact, undigested allergen.

Different categories of food allergy have been defined according to the immunological mechanisms involved in the allergic response: IgE-mediated, non-IgE-mediated, mixed IgE- and non-IgE-mediated food allergies, and oral allergy syndrome (OAS).

2.1.1. IgE-Mediated Food Allergy

The most common and most clearly understood form of food allergy is the IgE-dependent type, also known as 'immediate type I hypersensitivity.' The development of a food allergy begins with a process known as sensitization. During sensitization, inflammatory cytokines such as interleukin-25 (IL-

25), interleukin-33 (IL-33) and thymic stromal lymphopoietin (TSLP) are released, activating one class of antigen-presenting cells (APC), dendritic cells, which develop phenotypes normally acquired during pathogen defense while displaying fragments of food allergen proteins on their cell surface. These activated dendritic cells stimulate naive T cells, causing them to develop a T helper cell 2 (Th2) phenotype, which in turn promotes inflammatory signals. Some B and T lymphocytes also recognize the allergens displayed by the APCs through their cell surface receptors, activate, multiply, and differentiate, consequently producing food antigen-specific IgE (Anvari et al., 2018).

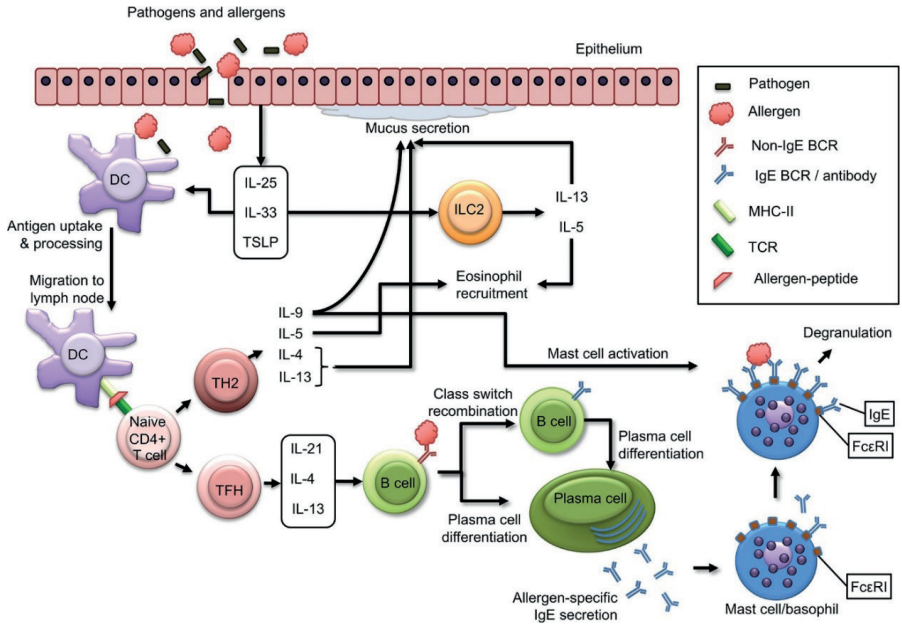

Figure 1.1: Molecular mechanisms of sensitization in IgE-mediated food allergy (Schoos et al., 2020). IL-25, IL-33 and TSLP are produced by epithelial cells due to contact with the allergen. These 'alarmins' stimulate dendritic cells to migrate to the lymph nodes, generate Th2 responses and trigger TFH (T follicular helper) cells. IL-24, IL-4 and IL-13 secreted by TFH cells stimulate B cells to generate allergen-specific IgE. These IgEs bind to FcRI receptors on mast cells and basophils, which can activate when re-exposed to the allergen.

In sensitized individuals, subsequent exposure to the allergenic protein triggers an allergic response, when it binds to allergen-specific IgE antibodies that stud the surface of granulocytes such as mast cells and basophils. This leads to the activation of these cells and the release of various chemical mediators, such as histamine, tryptase, platelet activating factor,

prostaglandins, and leukotrienes. The release of these chemical mediators triggers an inflammatory response, resulting in the characteristic symptoms of a food allergy (Figure 1) (Schoos et al., 2020).

2.1.2. Non-IgE-Mediated and Mixed IgE- & Non-IgE-Mediated Food Allergy

Another group of disorders, characterized by subacute or chronic inflammation in the gut in response to specific foods but with little or no IgE production, are known as 'non-IgE-mediated food allergies.' Symptoms are typically restricted to the gastrointestinal (GI) tract, present early in infancy and usually resolve by the age of 3-5 years (Zhang et al. 2021). The mechanisms triggering these conditions are not well understood, although activation of the innate immune response and/or Th2-type pro-inflammatory cytokines are implicated. Often allergen-specific IgE is not detected, although in some studies a low level of specific IgE was present and associated with more persistent symptoms (Caubet et al. 2014).

A "mixed" response - where some IgE production is observed but not thought to be the main driver of allergy - is also a characteristic of eosinophilic GI disorders, which are identified by excessive infiltration of eosinophils into specific parts of the GI tract, most commonly the esophagous (Eosinophilic Esopaghitis, EoE). Increased levels of Th2-type cytokines and disruption of the epithelial barrier are thought to underlie the pathology (Zhang et al. 2021).

2.1.3. Oral Allergy Syndrome

Oral allergy syndrome (OAS) is a common allergic response characterized by itching, swelling and pain in the lips, tongue, palate, ears and throat, which is triggered rapidly on consumption of specific foods, but usually resolves equally quickly. It is an IgE-mediated repsonse, but is often triggered by 'Type 2' allergens that are readily broken down by heat or digestive processes. Molecular studies have shown that OAS is caused by sensitization to a different allergen protein that happens to be structurally similar to those that then trigger the allergic response (Alessandri et al. 2020). The best known examples involve primary sensitization through airway exposure to tree pollen allergens, which have cross-reactivity with several protein families that are found in fresh fruits and vegetables. Therefore, exposure to one source of allergens by inhalation leads to cross-reactivity with the other. It should be noted that OAS may co-occur with one of the other types of food allergy described above (Alessandri et al. 2020).

2.2. The Role of Basophils

Basophils and mast cells are both granulocytes, a type of white blood cell that plays a role in the immune system's reaction to allergens and parasitic infections. They share common features such as surface IgE receptors and internal granules carrying a variety of chemical mediators, including histamine and heparin. While mast cells are resident in specific tissues, basophils are produced from bone marrow cells and circulate in the bloodstream. Basophils are comparatively rare in circulation, constituting only a small percentage of the total white blood cell population.

Basophils play an important role in allergic reactions, especially acute hypersensitivity reactions. When exposed to an allergen, basophils become activated due to the binding of allergen-specific IgE antibodies to their surface. This causes the granule contents, including histamine, to be released, leading to the symptoms of an allergic reaction (Siracusa et al., 2013). Histamine is a potent vasodilator that promotes blood vessel dilation and increased permeability, contributing to the characteristic symptoms of food allergy, such as itching, urticaria, and swelling. Other mediators released by basophils can further amplify the allergic response and recruit other immune cells such as eosinophils to the site of the allergic reaction (Kabashima et al., 2018).

Basophils can also interact with other immune cells, such as T cells and dendritic cells, to promote and amplify the immune response in food allergies. They can secrete cytokines, such as interleukin-4 (IL-4) and interleukin-13 (IL-13), which promote the differentiation of other immune cells into Th2-type inflammatory subtypes, increasing the development of allergic responses (Redrup, 1998).

3. Therapeutic Approaches in Food Allergy

3.1 Diagnosis

The diagnosis of food allergies involves a combination of medical evaluation, clinical history assessment, and specific diagnostic tests such as oral food challenge (OFC), skin prick test (SPT), basophil activation test (BAT), serum sIgE-level test and/or omics-based tests. Qualified healthcare professionals experienced in allergy management should consider the individual's clinical history, symptoms, and test results to make an accurate diagnosis and provide appropriate guidance for allergen avoidance and management strategies. (Sicherer & Sampson, 2014).

A detailed medical history is crucial in identifying potential food allergies. The healthcare providers ask about symptoms experienced after consuming specific foods, the timing and duration of symptoms, and any known triggers. They also inquire about personal or family history of allergies, asthma, eczema, or other allergic conditions. For example, the onset of atopic eczema within the first 6 months of life is linked to the emergence of food allergies to egg, peanut, and milk (Turnbull et al., 2014).

An elimination diet may be suggested, removing suspected allergenic foods from the diet to see if symptoms improve. This is typically performed under the guidance of a healthcare professional to avoid nutritional deficiencies. After a period of elimination, foods are gradually reintroduced one at a time to identify the specific food causing the allergic reaction. Additionally, keeping a detailed food diary can help track symptoms and identify potential patterns or correlations between certain foods and allergic reactions. However, it should be noted that medical history is unreliable and open to misperceptions since it depends on the patient's recollection of the events (Sampson, 1999).

In some cases, an OFC may be conducted under medical supervision. The double-blind placebo-controlled food challenge (DBPCFC) is accepted as the gold standard for food allergy diagnosis, and involves the controlled ingestion of a suspected allergenic food in increasing amounts to observe the development of allergic symptoms. However, due to the risk of anaphylaxis, the high cost, resource-intensive and time-consuming process, OFCs are practically used only in strictly necessary conditions (Turnbull et al., 2014).

SPT involves placing a small amount of allergenic extracts on the skin, usually the forearm or back. The skin is then gently pricked using a sterile lancet to allow the allergen to enter the top layers of the skin. If a person is allergic to the specific allergen, an allergic reaction will occur at the site, characterized by a raised bump or wheal (Schoos et al., 2020).

Blood tests, such as specific IgE (sIgE) tests or component-resolved diagnostics (CRD), measure the levels of allergen-specific IgE antibodies in the blood. These tests can provide an indication of sensitization to specific allergenic proteins. However, some sensitized individuals become tolerant to the original food allergen, so these tests are not definitive and should be used in conjunction with other diagnostic methods (Sicherer & Sampson, 2014).

Another test that can be carried out directly from blood samples is the Basophil Activation Test, in which the upregulation of cell surface marker CD63 on circulating basophils in response to increasing levels of allergen is

measured by flow cytometry (Santos et al. 2014). As a functional readout of the allergic response this approach can discriminate between allergic and tolerant individuals, but there is a need to establish standardized readouts and controls before it can be widely applied in clinical diagnostics.

3.2. Treatment

Currently, there are no medicines or treatments that can cure or protect against food allergies with the exception of Palforzia, an oral immunotherapy (OIT) agent for peanut allergy recently approved by the USA Food and Drug Administration (FDA). Other than that, the treatment of food allergies involves a combination of strict allergen avoidance, education of families and patients to follow the guidelines, emergency preparedness, and in certain circumstances, medical intervention (Hwang et al., 2022).

The primary strategy for managing food allergies is to avoid the foods that trigger an allergic reaction. This involves reading food labels carefully, being mindful of hidden or cross-reacting allergens, and taking precautions when dining out or eating at social events. Even so, according to a survey, almost 20% percent of children diagnosed with food allergy had to visit emergency departments due to food allergy related anaphylaxis each year in the United States (Gupta et al., 2018). Unfortunately, this strategy causes anxiety in children and families, as well as isolation at social gatherings and celebrations (Hwang et al., 2022).

Individuals with severe food allergies, especially those at risk of anaphylaxis, should be prepared for emergency situations. Carrying an epinephrine auto-injector, knowing when and how to use it, and having an emergency action plan in place are all part of this. Family members, friends, and caretakers should also be trained to recognize the signs of an allergic reaction and to deliver emergency treatment if necessary. Other medications may be prescribed to manage specific symptoms or provide relief during accidental exposure. Antihistamines can help alleviate mild to moderate allergic symptoms such as itching and hives, and corticosteroids reduce inflammation and suppress immune responses. However, it should be noted that these should not be relied on the food allergy management and priority should be given to the epinephrine injection (Sicherer & Sampson, 2014).

Immunotherapy is an emerging treatment option for some food allergies. Four types of immunotherapies currently under research are subcutaneous immunotherapy (SCIT), oral immunotherapy (OIT), sublingual immunotherapy (SLIT) and epicutaneous immunotherapy (EPIT). They all involve controlled exposure to small amounts of the allergenic food

over time, gradually increasing the dose to desensitize the immune system (Hwang et al., 2022).

Palforzia by Aimmune is the first OIT drug that has been approved by the FDA, to treat peanut allergy in children. It is given on a daily basis and comprises Peanut (*Arachis hypogaea*) Allergen powder-dnfp (PTAH), a formulation of defatted peanut flour with strictly determined levels of 3 key allergen proteins. Clinical trials reported that children and adolescents who received 300 mg PTAH daily for two years had higher tolerance to peanut ingestion and lower levels of serum peanut-specific IgE (psIgE) (Fernandez-Rivas et al., 2021). Although Palforzia has been shown to be effective in reducing peanut sensitivity, during the trial period 85% of participants suffered some adverse effects in their gastrointestinal tract and 81% in their respiratory tract (Vickery et al., 2018). Therefore, Palforzia can only be administered under the direction of a risk management program directed by certified healthcare providers.

4. MicroRNAs: a New Player in Food Allergy

4.1. Biosynthesis and Molecular Function of MicroRNA

MicroRNA (miRNA) is a class of small non-coding, single-stranded RNA molecules that are typically 18-24 nucleotides long and play a crucial role in post-transcriptional gene regulation in most eukaryotic cells. They are involved in diverse biological processes, including development, cell differentiation, metabolism, apoptosis, and response to stress. Dysregulation of miRNAs has been implicated in several diseases, including cancer, cardiovascular disorders, neurological conditions, and immune disorders (MacFarlene et al., 2010). Because of their regulatory functions and potential as diagnostic and/or therapeutic targets, miRNAs have received significant attention in biomedical research.

miRNAs can inhibit translation of specific messenger RNA (mRNA) molecules by binding to complementary target sequences within them, marking the resulting double-stranded RNA (dsRNA) duplex for degradation, thereby regulating the expression of genes. Elimination of dsRNA from the cell is a natural process known as RNA interference (RNAi), which has been conserved in many eukaryotes as a defense against RNA viruses (Xu et al., 2019). The biosynthesis of miRNAs is a multi-step process summarized in Figure 2.

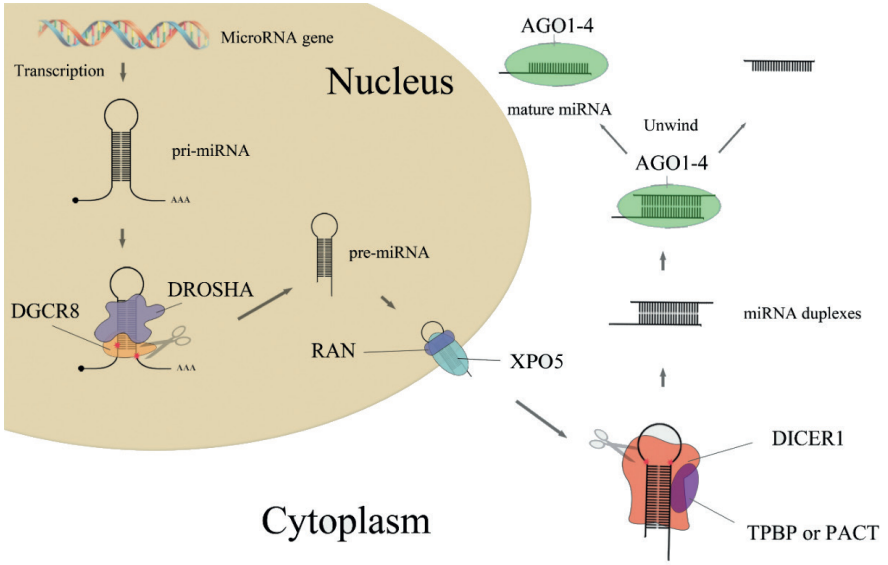

Figure 2. miRNA biosynthesis (adapted from He et al., 2016). Pri-miRNA is transcribed from a miRNA gene in the nucleus. DGCR8 (DiGeorge syndrome Critical Region 8) and DROSHA form the 'microprocessor complex.' XPO5 = Exportin 5, RAN = Ran small GTPase. DICER1 removes the hairpin structure, then the miRNA duplex is loaded onto the AGO protein. The miRISC complex is functional once one of the 2 miRNA strands is released.

Primary miRNAs (pri-miRNAs) are transcribed from miRNA genes in the nucleus by the same transcription machinery as protein-coding genes, giving them a 5' methylated cap and a 3' poly-A tail. However, they are usually much shorter (70-100 nt) and fold into a characteristic 'hairpin' structure. This hairpin allows recognition by a complex consisting of an RNA binding protein, DGCR8, and a ribonuclease III enzyme (RNase), Drosha, which trims both ends of the pri-miRNA to give a precursor miRNA (pre-miRNA) with a characteristic 2 nt 3′ overhang. In animal cells, pre-miRNAs are subsequently exported to the cytoplasm by the exportin 5 (XPO5)/Ran GTPase complex before being processed by Dicer, another type III RNase. Removal of the terminal loop by Dicer leaves a mature miRNA duplex that is recognized by Argonaute (AGO) proteins and incorporated into a miRNA-induced silencing complex (miRISC). The miRISC recognizes and binds to target mRNA through complementary miRNA response elements (MREs) (O'Brien et al., 2018).

4.2. Extracellular & Circulating miRNAs

Most miRNAs regulate gene transcription within the cell in which they were synthesized. However, in recent years an increasing number of miRNAs have been identified circulating in extracellular areas and are known as cell-free or circulating miRNAs. They act as intercellular messengers, participating in a variety of biological processes by regulating gene expression in recipient cells (Cui et al., 2019). They are found in various body fluids, including blood, serum, tears, urine, saliva, and breast milk (Salloum-Asfar et al., 2019).

Depending on the mechanism of release, circulating miRNAs are categorized into vesicle-associated and non-vesicle-associated miRNAs (Figure 3). Non-vesicle-associated circulating miRNAs constitute the majority of the extracellular miRNAs, predominantly in ribonucleoprotein complexes. Protein miRNA carriers such as Argonaute2 (AGO2), GW182, Nucleophosmin-1 (NPM1), and high-density lipoprotein (HDL) have been confirmed to protect and stabilize extracellular miRNAs. Alternatively, circulatory miRNAs can be transferred and targeted to distant tissues by encapsulation in exosomes or microvesicles. This involves an active loading process that uses adenosine triphosphate (ATP) as its energy source and may be related with selectivity and a high order of regulation in homeostasis. Several studies have suggested that miRNAs protected and shuttled by exosomes play crucial regulatory roles in cell-cell communication (Cui et al., 2019). miRNA dysregulation has been linked to a variety of human disorders, including cardiovascular disease, diabetes, allergic rhinitis, atopic dermatitis, asthma, and cancer (Hamam et al., 2017; Weidner at al., 2020). Their structural stability and resistance to RNase degradation has attracted interest in circulating miRNAs as potential non-invasive diagnostic biomarkers for several of these diseases (Mitchell et al., 2008).

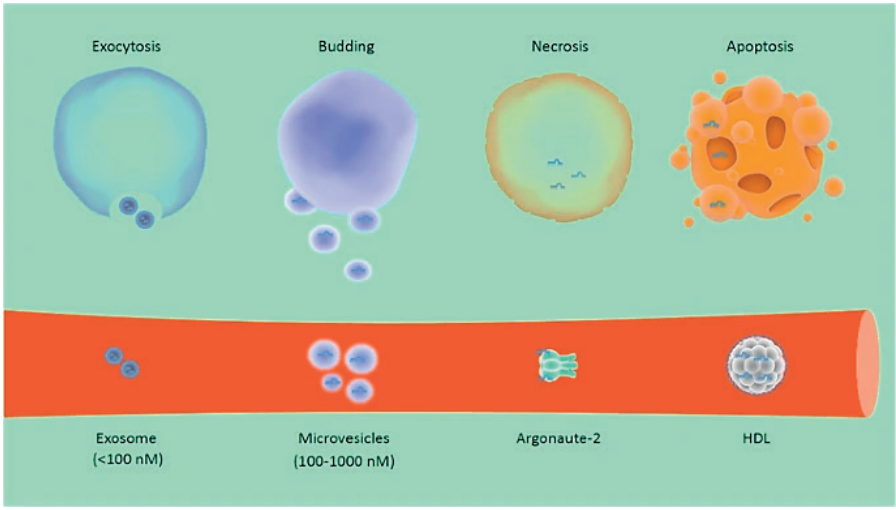

Figure 3. Carrier molecules of extracellular miRNAs and their probable formation mechanisms. Vesicle-associated extracellular miRNAs can be encapsulated in exosomes or microvesicles, while other circulating miRNAs are bound to carrier proteins. HDL = High Density Lipoprotein. First published in Hamam et al. (2017) and made available under Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/)

The study of the functions, mechanisms of action, and impacts of circulating miRNAs is still ongoing. Our present understanding is growing each year, bringing new insights into the nature of endogenous extracellular miRNAs and their potential applications in medical diseases (Salloum-Asfar et al., 2019).

4.3 Potential Roles of miRNA in the Development of Food Allergy

Several dozen human miRNAs have been correlated with the development and pathology of allergic diseases including atopic dermatitis, allergic rhinitis and asthma (reviewed by Weidner et al. 2020). In comparison miRNAs in food allergy are not widely researched, although some results are beginning to emerge. Furthermore, some studies suggest that non-human miRNAs found in food could be transferred to immune cells in the GI tract, presenting the intriguing possibility that cross-species RNA interactions might influence allergic responses.

4.3.1 Human Circulating miRNA Interactions in Food Allergy

Most studies of serum miRNA expression during food allergy have focused on their value as biomarkers to help predict the risk of a severe allergic reaction. For example, in a cohort of patients presenting with suspected anaphylactic shock, human miR-451a was found to be the serum biomarker most consistently upregulated in anaphylaxis, and also present at higher baseline levels in allergic patients than healthy controls (Francuzik et al. 2022). The patients from the aforementioned study were mostly allergic to insect stings, but research in children with acute peanut allergy found that both miR-21-3p and miR-487-3p were upregulated during anaphylaxis (Nunez-Borque et al. 2021). A similar study of peanut-allergic adults identified at least 15 serum miRNAs that increased after oral allergen challenge, although there was also high variability in their expression levels in non-allergic controls (Worm et al. 2022). miRNAs are also implicated in non-anaphylactic food allergies; profiling of miRNAs in esophageal biopsies from patients suffering from EoE again found upregulation of miR-21, along with miR-223 (Lu et al., 2012).

In addition to the allergic response, miRNA activity during the allergen sensitization phase must also be considered. For example, during the development of cow's milk allergy (CMA) in infants, it was observed that miR-193a-5p was down-regulated compared to healthy controls (D'Argenio et al. 2018). miR-193a-5p targets the IL-4 mRNA in T cells, and so is thought to suppress Th2-type responses. Accordingly, in a randomized controlled trial of infants with IgE-mediated CMA, the group that were fed with a formula designed to induce immune tolerance to casein showed significantly increased serum levels of miR-193a-5p (and also miR-128, miR-146a & miR155) and correspondingly reduced levels of IL-4, compared to patients receiving a neutral soya-based formula (Paparo et al. 2019).

These findings demonstrate that changes in serum miRNAs occur during both food allergy sensitization and allergic reactions. Those that are up-regulated may be useful as biomarkers, while supplementation to increase those that are down-regulated in allergic patients could be explored as a novel immunomodulatory treatment strategy.

4.3.2 Dietary miRNAs and Immune Regulation

Different species produce different miRNAs, but the RNAi mechanism is essentially conserved in all multicellular organisms. Therefore, it has been proposed that some miRNAs from ingested food could be absorbed by and inhibit gene expression in the consumer, modifying immune responses

(Cavalieri et al. 2016, Chin et al. 2016). For example, plant-derived-miRNAs inhibited T cell proliferation *in vitro* and reduced inflammatory responses in a mouse model of multiple sclerosis (Cavalieri et al. 2016). Plant miR-156a was also shown to have an anti-inflammatory effect in a model of atherosclerosis (Hou et al. 2018) while miR-156c & miR-159a isolated from dried tree nuts reduced inflammatory cytokine production *in vivo* in a mouse model of obesity (Aquilano et al. 2019). Thus, we hypothesize that ingested dietary miRNAs could have an effect on the development of food allergies.

Two question marks over the effects of dietary miRNAs is whether they are degraded during food processing and digestion, and how they could be absorbed by cells in the GI tract. However, in a similar manner to circulating miRNAs, recent studies have found that some exogenous miRNAs are packaged into exosome-like nanovesicles (Hirschi et al, 2015), protecting them from degradation and potentially facilitating uptake. Furthermore, it has recently been shown in mice that the transporter protein SIDT1 (SID1 Transmembrane family protein 1) is enriched in the plasma membrane of stomach epithelial cells and permits absorption of plant-derived mature miRNAs directly from the stomach (Chen et al. 2021). In this study, unpackaged miRNAs were protected from RNase degradation by the acidic environment in the stomach, and could subsequently be detected in mouse serum and transmitted to other tissues.

5. Conclusions and Future Work

Food allergy is a widespread and growing public health problem worldwide, although this label actually encompasses a set of different but related immune disorders, with implications ranging from mild to life-threatening. Many of the key biomolecules and cell types involved in food allergies are now known, but the specific molecular triggers that cause some individuals to become sensitized to allergen proteins while the other consumers do not are still unclear. Meanwhile for those who do develop a severe food allergy and their family members, the difficulty of reaching an accurate diagnosis and the lack of effective treatment has a significant impact on quality of life.

In this chapter, we have highlighted the potential value of miRNAs as novel biomarkers and therapeutic agents in food allergy. They are relatively easy to synthesize and amplify, and utilize the body's own gene regulation mechanisms. Furthermore, a number of different miRNA mimics and inhibitors are already in clinical trials for use in the diagnosis or treatment of cardiovascular diseases, hepatitis and cancer (Kim & Croce, 2023).

The findings described above show that some miRNAs are differentially regulated during food allergy, while at least in mouse models, dietary miRNAs have been demonstrated to have anti-inflammatory activity in and beyond the GI tract. However, just as the precise molecular mechanisms of sensitization in food allergy are not known, neither are the points at which miRNAs could have a clinical impact. Therefore, future research directions should include:

i) Identifying which miRNAs are present at biologically relevant concentrations in allergenic foodstuffs, and whether they can be absorbed through the GI tract

ii) Defining the key genetic and signalling pathways activated during food allergy, including genes in these pathways that could be miRNA targets

iii) Demonstrating the functional effect of possible miRNA-target interactions *in vitro* in relevant cell types (e.g. T cells, basophils), followed by animal models.

While the primary aim of this research would be to develop miRNA-based diagnostics and therapeutics, elucidating the mechanisms by which they affect food allergies also promises to reveal new cellular and molecular targets for intervention.

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