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Research Progress on MicroRNAs Involved in the Regulation of Chicken Diseases

Yang Wang

College of Animal Science and Technology, Qingdao Agricultural University, Qingdao, 266109, P.R. China

MicroRNAs (miRNAs) are small, non-coding RNA molecules that inhibit protein translation from target mRNAs. Accumulating evidence suggests that miRNAs can regulate a broad range of biological pathways, including cell differentiation, apoptosis, and carcinogenesis. With the development of miRNAs, the investigation of miRNA functions has emerged as a hot research field. Due to the intensive farming in recent decades, chickens are easily influenced by various pathogen transmissions, and this has resulted in large economic losses. Recent reports have shown that miRNAs can play critical roles in the regulation of chicken diseases. Therefore, the aim of this review is to briefly discuss the current knowledge regarding the effects of miRNAs on chickens suffering from common viral diseases, mycoplasmosis, necrotic enteritis, and ovarian tumors. Additionally, the detailed targets of miRNAs and their possible functions are also summarized. This review intends to highlight the key role of miRNAs in regard to chickens and presents the possibility of improving chicken disease resistance through the regulation of miRNAs.

Key words: chicken, miRNA, necrotic enteritis, ovarian tumor, virus disease

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Introduction

Poultry rearing is an industry that is superior to other sectors in agriculture, and recently, this industry has undergone tremendous development (Uddin *et al*., 2010; Landoni and Albarello, 2015). Commercial poultry production is a very intensive animal agricultural system, and one poultry house or barn can contain as many as 100,000 commercial layers or broilers (Landoni and Albarellos, 2015). Given this, mortality of chickens due to various infectious and non-infectious diseases is emerging as the major constraint against profitable poultry production. For example, Marek's disease (MD) and lymphoid leukosis (LL) are both viral diseases that affect chickens to cause tumors and high mortality (Kreager, 1998). Infectious bursal disease (IBD) is one of the most prevalent infectious virus diseases, causing bursal necrosis and immunosuppression that lead to severe damage to the immune system in chickens (Xu *et al*., 2019). Avian influenza (AI) is also a highly contagious viral disease that is responsible serious animal health crises that result in high fatality rates in poultry worldwide (Paul *et al*., 2019). Avian mycoplasmosis is another disease that can cause chronic respiratory disease (CRD) in chickens (Ley, 2008), and necrotic enteritis (NE) is a ubiquitous poultry disease caused by *Clostridium perfringens* that affects the intestines of chickens, resulting in major impacts on performance (Whelan *et al*., 2018). Given these examples and the prevalence of diseases affecting chickens, it is clear that disease control and prevention at all levels must be a major focus for the poultry industry.

MicroRNAs (miRNAs) are small, non-coding RNAs. In animals, miRNAs are approximately 22 nucleotides in length, and they play vital roles in almost all developmental and pathological pathways (Jansson and Lund, 2012). MiRNAs play an important role in mRNA degradation, where they can bind to the three prime untranslated regions (3'-UTR) of various mRNA transcripts to reduce translation (Bartel, 2004). It has been reported that within a given genome, 20%–30% of genes are regulated by miRNA (Enright *et al*., 2003), and a single miRNA may target more than 100 mRNAs (Friedman *et al*., 2009). Therefore, identification of miRNA functions in different organisms is a critical step to facilitate our understanding of genome organization, genome biology, and evolution (Carrington and Ambros, 2003).

In the past ten years, miRNAs have been discovered to possess multiple functions in chickens, including the regulation of development and various growth processes

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Correspondence: Dr. Yang Wang, College of Animal Science and Technology, Qingdao Agricultural University, Qingdao, 266109, P.R. China. (E-mail: yangwang@qau.edu.cn)

(Bannister *et al*., 2011; Luo *et al*., 2014). Additionally, in recent decades researchers have reported that miRNAs play diverse roles in chicken diseases and immune system functions (Ahanda *et al*., 2009; Hicks *et al*., 2009; Dinh *et al*., 2014; Liu *et al*., 2016). The majority of these studies investigated the possible mRNAs that could be targeted by a given miRNA and the underlying mechanisms responsible for the observed effects. Thus, this review aims to introduce the functions of miRNAs in the context of chickens, and particular focus is placed on the advances related to the impact of miRNAs on various diseases that affect chickens. The identification of distinct miRNA expression patterns and the roles of those dysregulated miRNAs in the context of pathological conditions that affect chickens will broaden our understanding of how miRNAs regulate the chicken immune system and reveal potential therapeutic targets for the treatment of miRNA-related diseases.

MiRNA Biogenesis

The biogenesis of miRNA has been elaborately reviewed in the existing literature (Bushati and Cohen, 2007; Lin and Gregory, 2015). Briefly, miRNA begins from a primary transcript, termed the pri-miRNA, that is typically a long mRNA that is thousands of nucleotides in length and transcribed by RNA polymerase II (only a few miRNAs are transcribed by RNA polymerase III) (Magenta *et al*., 2013). Then, the pri-miRNA is processed by the nuclear RNase III Drosha-DGCR8 complex into an approximately 65 nucleotide hairpin precursor miRNA (pre-miRNA) (Denli *et al*., 2004). Next, the pre-miRNAs are exported from the nucleus into the cytoplasm by exportin 5 and further processed by DICER1, an RNase III enzyme that measures from the 5' and 3' ends of the pre-miRNA to form the mature ~22 nucleotide miRNA: miRNA* (the complementary strand of miRNA) duplex (Lin and Gregory, 2015). The miRNA* is typically degraded, while the mature miRNA is incorporated into the RNA-induced silencing complex (RISC) (Bartel, 2004). MiRNAs play an important role in mRNA degradation, as they can bind to the 3'-UTR of mRNA transcripts to reduce translation (Bartel, 2004) (Fig. 1).

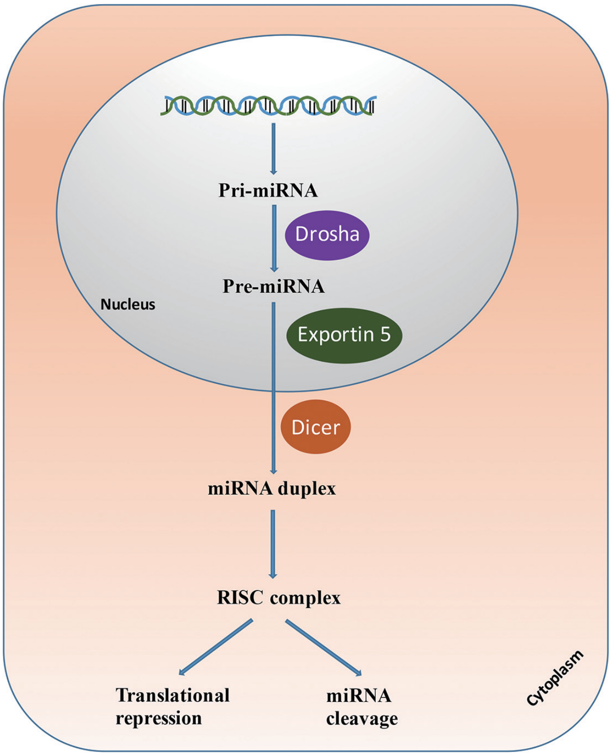

Fig. 1. **miRNA biogenesis paradigm**

MiRNAs Involved in Chicken Virus Diseases

The interactions among miRNAs and viruses are highly complicated. In some instances, viruses may encode their own miRNA to facilitate their pathogenesis (Muylkens *et al*., 2010). In other instances, however, host miRNAs can directly target viral RNAs during infections or they can regulate various immune responses to inhibit virus replication (Wang *et al*., 2010; Ingle *et al*., 2015; Wang *et al*., 2018).

MiRNA and Infectious Bursal Disease

Infectious bursal disease (IBD) is an acute, highly contagious disease in young chickens (Pitcovski *et al*., 2003). Infectious bursal disease virus (IBDV), which destroys B-lymphocyte precursors and causes a high degree of immunosuppression, is considered to be one of the major threats to the poultry industry (Wang *et al*., 2018). IBDV contains two segments of double-stranded RNA (A and B) (Hirai and Shimakura, 1974, Azad *et al*., 1985). Segment A possesses two partially overlapping open reading frames (ORFs) (Kibenge *et al*., 1991), while segment B encodes an RNA-dependent RNA polymerase, viral protein 1 (VP1) (von Einem *et al*., 2004). Recent studies have revealed that miRNAs may suppress IBDV replication by targeting the viral genome segments and host cellular *SOCSs* (suppressor of cytokine signaling). SOCSs are considered to be key physiological regulators of the immune system and can negatively regulate type I interferon (IFN) (Fu *et al*., 2017) that allows host cells to recognize viral dsRNA through the action of TLR3 or RIG-I molecules (Li *et al*., 2013) to combat viral infection. For example, according to Wang *et al*., gga-miR-155 could inhibit IBDV replication and enhance the expression of *IFN* via targeting *SOCS1* and *TANK* (TNF receptor-associated factor family member-associated NF-κB activator) in response to IBDV infection (Table 1, Wang *et al*., 2018). Additionally, gga-miR-130b could suppress IBDV replication via targeting a specific sequence of IBDV segment A and enhancing the expression of IFN-β by binding to the host *SOCS5* (Table 1, Fu *et al*., 2017). Similarly, gga-miR-454 was found to inhibit IBDV replication by binding to a specific sequence of IBDV segment B and enhancing the expression of IFN-β by targeting cellular *SOCS6* (Table 1, Fu *et al*., 2018). Additionally, gga-miR-21 could target viral genomic RNA and inhibit *VP1* translation to lower IBDV replication (Table 1, Wang *et al*., 2013a).

In contrast, gga-miR-9* negatively regulated the host antiviral innate immune response by suppressing type I IFN production by targeting *IRF2* (interferon regulatory factor 2), an important factor that functions in IFN antiviral signal transduction pathways, to promote IBDV replication (Table 1, Ouyang *et al*., 2015). gga-miR-2127 overexpression also promoted IBDV replication and could down-regulate the expression of antiviral innate immunity genes by targeting *p53* (Table 1, Ouyang *et al*., 2017). Additionally, gga-miR-142-5p targeted *chMDA5* (chicken melanoma differentiation-associated gene 5), which encodes a protein that recognizes RNA viral infections and initiates an antiviral innate immune response, to promote IBDV replication in an IRF7-dependent pathway (Table 1, Ouyang *et al*., 2018).

MiRNA and Marek's Disease

Marek's disease (MD) is a type of chicken lymphoma that is caused by Marek's disease virus 1 (MDV1), an α-herpesvirus (Osterrieder *et al*., 2006), that results in immunosuppression, neurological disorders, and rapid-onset CD4 + T-cell lymphoma (Biggs, 1997). MD was the first tumor disease that could be prevented by vaccination, and it provides an important animal model for the study of viral cancer development and immunity (Osterrieder *et al*., 2006).

In recent decades, numerous studies have been performed to investigate the involvement of miRNAs (both host and MDV miRNAs) in MD tumorigenesis. Through the use of microarrays and qRT-PCR analysis, two clades of chicken miRNAs were found to be increased in splenic tumors and non-tumorous spleen tissues derived from MDV1-infected chickens (Li *et al*., 2014a). Additionally, chicken lines 6_3 and 7_2 are two highly inbred lines of specific-pathogen-free white leghorn chickens that are resistant or susceptible, respectively, to MD tumors. According to Tian *et al*. (2012), gga-miR-15b, which targets *ATF2* (activating transcription factor 2), was reduced in MDV-infected susceptible chicken splenic tumors. To further understand the potential functions of miRNAs in MDV resistance and susceptibility, the expression of ATF2, a protein that can interact with MDV oncogene *Meq*, was determined. The results of this study revealed that the protein level of ATF2 was significantly increased in infected line 7_2 chickens compared to that of MDV-free chickens, while its expression was stable in line 6_3 chicken before and after MDV challenge. Chicken ATF2 was observed to form a heterodimer with c-Jun, the MDV oncogene *Meq*, and other b-ZIP proteins (Huguier *et al*., 1998; Levy *et al*., 2003; Osterrieder *et al*., 2006). It has been reported that ATF2 exerts tumor suppressor or oncogene activities in different cancers by cooperation with other tumor suppressors or oncogenes (Bhoumik *et al*., 2004; Bhoumik and Ronai, 2008). As the MDV oncogene *Meq* was highly expressed in line 7_2 after MDV infection, ATF2 likely formed a heterodimer with *Meq* to promote MD development, and in chicken line 6_3, due to the absence of *Meq* after MDV exposure, ATF2 may cooperate with c-Jun to facilitate distinct functions, similar to observations in line 7_2. Taken together, the results of this study suggested that gga-miR-15b may target *ATF2* to regulate MD resistance/susceptibility (Tian *et al*., 2012). Recently, gga-miR-219b was found to be decreased in MDV-induced lymphoma. Additionally, gga-miR-219b was demonstrated to target *BCL11B* (B-cell chronic lymphocytic/lymphoma 11B), a gene that not only plays an important role in thymocyte development but has also been implicated in lymphoproliferative diseases (Grabarczyk *et al*., 2007; Gutierrez *et al*., 2011). The knockdown of *BCL11B* also reduced the proliferation, migration, and invasion of the Marek's disease tumor cell MSB1. The inhibition of gga-miR-219b was further found to down-regulate the expression of the MDV oncogene *Meq*; however, *BCL11B* knockdown significantly induced *Meq* expression (Table 1, Zhao *et al*., 2017a). In other studies, gga-miR-26a, -181a, and -130a were similarly reported to inhibit the proliferation of MD lymphoid cells by *NEK6* (never in mitosis gene A-related kinase 6), *MYBL1* (v-myb myeloblastosis viral oncogene homolog-like 1), and *HOXA3* (homeobox A3), respectively (Table 1, Li *et al*., 2014b; Lian *et al*., 2015; Han *et al*., 2016). Additionally, it is known that miRNAs can be packaged into exosomes, a subset of extracellular vesicles that are of 30−150 nm in diameter and secreted by various cell types. Thus, exosome-mediated transport of miRNAs may provide a novel mechanism of intercellular gene regulation. According to Nath Neerukonda *et al*., serum exosomes were isolated from MDV-infected chickens that were either vaccinated against MD or unvaccinated and bearing MDV-induced tumors. When detecting the expression of various miRNAs within exosomes, researchers observed that exosomes from vaccinated chickens exhibited greater expression of tumor suppressor miRNA (gga-miR-146b) and less expression of oncomiR (gga-miR-21) compared to those obtained from unvaccinated controls. Thus, gga-miR-146b and -21 may serve as serum exosome biomarkers for vaccine-induced protection and for MD tumors, respectively (Nath Neerukonda *et al*., 2019).

In addition to animals, herpesviruses also encode miRNAs. Early in 2006, Burnside and colleagues found that eight miRNAs were encoded by MDV. Five of these miRNAs flank the *Meq* oncogene, and three of them map to the latency-associated transcript (LAT) region of the virus genome (Burnside *et al*., 2006). Later, they also identified an additional seven miRNAs and '*' strands by deep sequencing of MDV1 infected CEF cells (Burnside *et al*., 2008). Currently, twenty-six MDV-1-encoded miRNAs processed from fourteen pre-miRNAs (MDV1-miR-M31 and MDV1-miR-M1 to MDV1-miR-M13) have been identified (http://microrna.sanger.ac.uk). All of the MDV-1-encoded miRNAs are focused within three gene clusters, including the Meq cluster, the LAT cluster, and the Mid cluster. The first cluster of MDV1-miRNAs, including MDV1-miR-M2, -M3, -M4, -M5, -M9 and -M12, is antisense to the MDV-1gene R-LORF8 and located adjacent to the *Meq* oncogene (Luo *et al*., 2010). MDV1-miR-M4 is a functional ortholog of miR-155 (Zhao *et al*., 2009). Recently, reports have indicated

Table 1. **Validated targets of miRNA relevant to the immunity of chickens**

miRNA	immune function	Tissue/ cell	Targets	Reference
gga-miR-155	inhibits IBDV replication	DF-1 cell	*SOCS1, TANK*	Wang *et al*., 2018
gga-miR-130b	inhibits IBDV replication	DF-1 cell	*SOCS5,* the specific sequence of IBDV segment A	Fu *et al*., 2017
gga-miR-454	inhibits IBDV replication	DF-1 cell	*SOCS6,* the specific sequence of IBDV segment B	Fu *et al*., 2018
gga-miR-21	inhibits IBDV replication	DF-1 cell	*VP1* of IBDV genome	Wang *et al*., 2013a
gga-miR-9*	promotes IBDV replication	DF-1 cell	*IRF2*	Ouyang *et al*., 2015
gga-miR-2127	promotes IBDV replication	DF-1 cell	*p53*	Ouyang *et al*., 2017
gga-miR-142-5p	promotes IBDV replication	DT40 cell	*chMDA5*	Ouyang *et al*., 2018
gga-miR-15b	regulates MDV1-induced tumorigenesis	spleen	*ATF2*	Tian *et al*., 2012
gga-miR-26a	suppresses MD lymphoma cell proliferation	MSB-1 cell	*NEK6*	Li *et al*., 2014b
gga-miR-181a	suppresses MD lymphoma cell proliferation	MSB-1 cell	*MYBL1*	Lian *et al*., 2015
gg-miR-130a	suppresses MD lymphoma cell proliferation	MSB-1 cell	*HOXA3*	Han *et al*., 2016
gga-miR-219b	suppresses MD lymphoma cell proliferation, migration and invasion	MSB-1 cell	*BCL11B*	Zhao *et al*., 2017a
MDV1-miR-M4-5p	promotes MDV1-induced tumorigenesis	CEF and DF-1 cells	*LTBP1, hnRNPAB*	Chi *et al*., 2015; Dang *et al*., 2017
MDV1-miR-M4-5p	modulates immune response to MDV infection	DF-1, MDCC-MSB-1 and MDCC-54-O cell	*GPM6B, RREB1, c-Myb, MAP3-K7IP2, PU.1, C/EBP*	Muylkens *et al*., 2010
gga-miR-485	inhibits H5N1 replication and dampens RIG-I-dependent inflammatory response	HEK 293T cell, A549 cell, MCF7 cell	*PB1* of H5N1 and *RIG-I* of host	Ingle *et al*., 2015
gga-miR-1249	inhibits H1N1 replication	HEK 293T cell, A549 cell, MDCK cell	*PB2* of H1N1	Wang *et al*., 2017
gga-miR-375	suppresses proliferation and promotes apoptosis of cells infected with ALV-J	DF-1 cell	*YAP1*	Li *et al*., 2014c
gga-miR-221 and -222	promotes proliferation and inhibits apoptosis of cells infected with ALV-J	DF-1 cell	*BMF*	Dai *et al*., 2015
gga-miR-1650	inhibits ALV-J replication and infection	DF-1 cell	*ALV-J genome*	Wang *et al*., 2013b
gga-miR-23b	inhibits ALV-J replication and infection	chicken spleen, DF-1 cell and HD11 cell	*IRF1*	Li *et al*., 2015
gga-miR-34b-5p	promotes ALV-J-infected cell proliferation and ALV-J replication	DF-1 cell	*MDA5*	Li *et al*., 2017
gga-miR-101-3p	suppresses the proliferation of chicken embryonic fibroblast infected with MG	DF-1 cell	*EZH2*	Chen *et al*., 2015
gga-miR-99a	suppresses the proliferation of chicken embryonic fibroblast infected with MG	DF-1 cell	*SMARCA5*	Zhao *et al*., 2017c

Table 1. **Validated targets of miRNA relevant to the immunity of chickens (continued)**

miRNA	immune function	Tissue/ cell	Targets	Reference
gga-miR-19a	activates NF-κB signaling pathway, promotes TNF-α expression and enhances cell cycle progression and proliferation of cells infected with MG	DF-1 cell	*ZMYND11*	Hu *et al*., 2016
gga-miR-130b-3p	promotes MG-infected cell proliferation	DF-1 cell	*PTEN*	Yuan *et al*., 2018
gga-miR-146c	activates TLR6/MyD88/NF-κB signaling pathway and promotes proliferation by inhibiting apoptosis of cells infected with MG	DF-1 cell	*MMP16*	Zhang *et al*., 2019
gga-miR-1615	regulates ovarian tumorigenesis	chicken ovary	*AvBD-11*	Lim *et al*., 2013

that MDV1-miR-M4-5p could induce the over-expression of the oncogene *c-Myc* by targeting *LTBP 1* (latent TGF-β binding protein 1) and inactivating the TGF-β signaling pathway during MDV1-infection (Table 1, Chi *et al*., 2015). Further study revealed that MDV1-miR-M4-5p could also target chicken *hnRNPAB* (heterogeneous nuclear ribonucleoprotein AB) to promote proliferation of both primary CEF and transformed chicken fibroblast DF-1 cells (Table 1, Dang *et al*., 2017). Additionally, using viruses modified by reverse genetics of the infectious bacterial artificial chromosome (BAC) clone of the oncogenic RB-1B strain of MDV, Zhao and colleagues demonstrated that the deletion of the six-miRNA cluster 1 from the viral genome abolished the oncogenicity of MDA. This loss of oncogenicity may be due to MDV1-miR-M4, as its deletion or a 2-nucleotide mutation within its seed region was sufficient to inhibit the induction of lymphomas. This role was further confirmed through the rescue of oncogenic phenotype by revertant viruses that expressed either the miR-M4 or the cellular homolog gga-miR-155 (Zhao *et al*., 2011). Using luciferase reporter assays, Muylkens and colleagues found that MDV1-miR-M4-5p could target the 3'UTR of *GPM6B* (glycoprotein M6-b), *RREB1* (Ras-responsive element-binding protein 1), *c-Myb*, *MAP3-k7ip2* (mitogen-activated protein kinase kinase kinase 7-interacting protein 2), *PU.1*, and *C/EBP* (CCAAT-enhancer-binding proteins). Additionally, MDV1-miR-M4-5p specifically inhibited the translation of two viral proteins (UL28 and UL32) that are involved in the cleavage and packaging of herpesvirus DNA (Table 1, Muylkens *et al*., 2010).

MiRNA and Avian Influenza

Avian influenza, also known as "Bird Flu", is one of the most severe respiratory viral infectious diseases of this decade (Koparde and Singh, 2011). Avian influenza virus (AIV) is a type A virus of the family *Orthomyxoviridae*. Although wetland birds such as wild ducks, gulls, and shorebirds are the natural hosts of AIV, this virus can also infect poultry birds, resulting in significant economic losses and an increased threat to public health due to the potential for host jumping from animals to humans (Webby and Webster, 2003). MiRNAs have been found to regulate AIV replication during infection. In chickens, when the miRNA expressions between H5N3 infected and non-infected commercial SPF layer chickens were compared at 4 days post-infection, 73 and 36 miRNAs were differentially expressed in lung and trachea, respectively, and there were more miRNAs highly expressed in non-infected tissues (Wang *et al*., 2009). In subsequent experiments examining broilers, however, a greater number of miRNAs were highly expressed in infected lungs than in non-infected lungs, indicating that the regulatory mechanism of the host response to AIV infection mediated by miRNAs may be different between layers and broilers (Wang *et al*., 2012). In another study, 1004 miRNAs were obtained from H9N2-infected and non-infected chicken embryo fibroblasts, and 48 miRNAs were expressed differently between the two groups. These miRNAs were predicted to target immune response-related genes (Peng *et al*., 2015). In recent years, experiments using mammalian cells have demonstrated that host gga-miR-1249 targets the H5N1 and H1N1 gene *PB2* that encodes an RNA polymerase required for viral replication to suppress virus replication (Table 1, Wang *et al*., 2017). Similarly, gga-miR-485 was found to bind to the H5N1 gene *PB1* to inhibit H5N1 replication, and gga-miR-485 could also target the host gene *RIG-I* (retinoic acid-inducible gene I). Basal amounts of RIG-I protein detect viral nucleic acids in the cytosol and induce type I IFNs. In turn, these IFNs increase the expression of IFN-stimulatory genes, including *RIG-I*. Thus, gga-miR-485 could suppress the RIG-I-dependent expression of type I and type III IFNs in H5N1-infected cells and dampen the RIG-I-dependent inflammatory response (Table 1, Ingle *et al*., 2015).

MiRNA and Subgroup J Avian Leucosis Virus

Avian leukosis viruses (ALVs) are a group of avian retroviruses that induce tumors in chickens (Liu *et al*., 2011). Chicken ALVs are classified into six subgroups (A-E and J). ALV-J, which primarily induces myeloid leukosis, causes more serious damage than that caused by the other virus subgroups (Gao *et al*., 2012). To broaden our understanding of the molecular mechanisms that occur during ALV-J infection, efforts have been made to examine the miRNA expression patterns in chickens that are infected with ALV-J. Through the use of chicken dendritic cells (DC), ALV-J was found to induce apoptosis during the early infection stages. MiRNA sequencing data from ALV-J-infected and -uninfected DCs revealed 122 differentially expressed miRNAs (115 that were up-regulated and 7 exhibiting down-regulation after injection). Through GO analysis, the target genes of these miRNAs, including gga-miR-204, -211, -221 and -6651, were found to be involved in the processes indicated by the 'antigen processing and presentation of exogenous peptides' and 'apoptosis' GO terms (Liu *et al*., 2016). Using miRNA microarray analysis, Li and colleagues found that in 10-week-old chickens, 12 miRNAs were differentially expressed within the livers of ALV-J-uninfected and -infected chickens. The expression levels of gga-mir-221, -222, -1456, -1704, -1777, -1790, and -2127 were up-regulated by ALV-J infection, while the expression levels of gga-let-7b, -let-7i, -125b, -375, and -458 were significantly down-regulated (Li *et al*., 2012). Among these differentially expressed miRNAs, gga-miR-375 overexpression was observed to decrease DF-1 cell proliferation and promote DF-1 apoptosis. Through prediction and dual luciferase reporter assays, gga-miR-375 was confirmed to target *YAP1* (yes-associated protein 1), an effector of Hippo signaling, that restrains cell proliferation and promotes apoptosis to influence normal cell fate and tumorigenesis (Harvey and Tapon, 2007; Saucedo and Edgar, 2007). Additionally, *in vivo* assays further indicated that gga-miR-375 was decreased in chicken livers 20 days after ALV-J infection. Simultaneously, the expression levels of *cyclin E* and *DIAP1* (drosophila inhibitor of apoptosis protein 1) were up-regulated after infection. DIAP1 functions in the early embryo to inhibit apoptosis (Yoo *et al*., 2000). Cyclin E, an important regulator of cell cycle progression, was reported to be increased in the majority of breast tumor tissues and in most leukemia solid tumors (Keyomarsi *et al*., 1994). Thus, the above findings indicate that gga-miR-375 functions as a tumor suppressor and plays an important role in inhibiting ALV-J tumorigenesis (Table1, Li *et al*., 2014c). In contrast, in their later study researchers discovered that gga-miR-221 and -222 may be tumor formation-relevant genes that could contribute to tumorigenesis during ALV-J infection by targeting *BMF* (BCL-2 modifying factor), a gene that exhibits pro-apoptotic function (Table1, Dai *et al*., 2015).

In addition to control tumorigenesis, chicken miRNAs can also regulate ALV replication directly. The genome of ALV-J is composed of three genes, including *gag*, *pol*, and *env* (Li *et al*., 2015). Wang and colleagues revealed that gga-miR-1650 could target the 5'UTR of ALV-J, and gga-miR-1650 overexpression could decrease gag protein expression and inhibit ALV-J replication (Table 1, Wang *et al*., 2013b). The gp85 protein is a viral envelope polypeptide that is encoded by the *env* gene within the ALV-J genome. As reported, gga-miR-23b expression was up-regulated in ALV-infected chicken spleens and could target *IRF1* (interferon regulatory factor 1), a critical regulatory protein of the inflammatory response that functions as a tumor suppressor and is involved in cell cycle progression and apoptosis (Hong *et al*., 2013). Additionally, gga-miR-23b overexpression resulted in increased gp85 expression, while *IRF1* overexpression caused a decrease in gp85 level. These results indicate that gga-miR-23b promoted ALV-J replication by targeting *IRF1* and by affecting gp85 expression (Table 1, Li *et al*., 2015). gga-miR-34b-5p could also target *MDA5* (melanoma differentiation-associated gene 5), a gene that encodes a protein that can detect ALV-J infection and trigger the MDA5 signaling pathway, to promote ALV-J-infected cell proliferation. Additionally, *MDA5* overexpression significantly decreased env levels and ALV-J virion secretion, while gga-miR-34b-5p overexpression elevated env protein expression after ALV-J infection to induce ALV-J replication (Table 1, Li *et al*., 2017).

MiRNA Involved in Avian Mycoplasmosis

Mycoplasma, an important prokaryote, can infect humans and a wide range of economically important livestock species (Hu *et al*., 2016; Nicholas and Ayling, 2016). *Mycoplasma gallisepticum* (MG) is one of the most important mycoplasmas that is frequently associated with avian CRD (Ley, 2008), which causes severe inflammation in the tracheas and lungs of chickens and turkeys (Davidson *et al*., 1982; Stipkovits *et al*., 2012). Recently, several studies have suggested that miRNAs play a key role in MG infection in chicken. By deep sequencing, Zhao and colleagues found that miRNAs are associated with MG infection in chicken lungs at 3 and 10 days post-infection. They found 45 and 68 differentially expressed miRNAs at 3 and 10 days, and these miRNAs targeted 6290 and 7181 genes, respectively. GO, KEGG, miRNA-GO-network, path-net, and gene-net analyses determined that the altered miRNAs regulated a large number of genes that function throughout various signaling pathways including the MAPK pathway, the focal adhesion regulatory pathways, the Wnt pathway, and the JAK/STAT pathway (Zhao *et al*., 2017b). gga-miR-101-3p was found to be up-regulated in the lungs of MG-infected chicken embryos, and gga-miR-101-3p could target and inhibit the host *EZH2* (enhancer of zeste homolog 2), a growth suppressor gene involved in the regulation of cell cycle progression and proliferation, to suppress the proliferation of chicken embryonic fibroblasts by blocking the G1-to-S phase transition (Chen *et al*., 2015). Additionally, *SMARCA5* (SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily A, member 5) is a member of the SWI/SNF family that exhibits ATPase and helicase activities. It has also been reported that SMARCA5 plays an important role in promoting cell proliferation both *in vitro* and *in vivo* (Stopka *et al*., 2000; Gigek *et al*., 2011). According to a study by Zhao *et al*., gga-miR-99a was significantly down-regulated in the lungs of MG-infected chicken embryos and could target *SMARCA5* to repress the proliferation of DF-1 cells by inhibiting the transition from the G1 phase to the S and G2 phases (Zhao *et al*., 2017c). Additionally, gga-miR-19a, which targeted the host *ZMYND11* (zinc-finger protein, MYND-type containing 11), was found to be up-regulated both in chicken embryonic lungs and in MG-infected DF-1 cells to activate the NF-κB signaling pathway, ultimately promoting TNF-α expression and enhancing cell cycle progression and proliferation to defend against MG infection (Table 1, Hu *et al*., 2016). gga-miR-130b-3p was also markedly up-regulated in MG-infected DF-1 cells. Luciferase reporter assays confirmed that *PTEN* (phosphatase and tensin homolog deleted on chromosome ten) was a direct target of gga-miR-130b-3p. As one of the most important tumor suppressors, PTEN is a phosphatase that exhibits both protein and lipid activities, and this protein plays a central role in various cellular functions and in the immune response (Di Cristofano and Pandolfi, 2000). Studies also demonstrated that PTEN exerted its inhibitory effects against cell proliferation by blocking cell cycle progression at the G1 phase by facilitating the down-regulation of cyclins and CDKs proteins (Moon *et al*., 2004). Thus, the overexpression of gga-miR-130b-3p remarkably promoted MG-infected DF-1 proliferation by binding to *PTEN* (Table 1, Yuan *et al*., 2018). Matrix metalloproteins (MMPs), a family of zinc-dependent endopeptidases, are important for a number pathological process such as inflammation, cardiovascular disease, and cancer (Huang *et al*., 2017; Mali *et al*., 2017). A recent study revealed that gga-miR-146c was up-regulated in response to MG infection, and *MMP16* was validated as the target gene of gga-miR-146c (Zhang *et al*., 2019). Additionally, using loss- and gain-of-function approaches, Zhang and colleagues also found that gga-miR-146c participated in the activation of the TLR6/MyD88/NF-κB pathway. It was also observed that gga-miR-146c could decrease cell apoptosis and stimulate DF-1 proliferation after MG infection. Taken together, these findings indicated that gga-miR-146c defended against host MG infection by inhibiting *MMP16* expression, activating the TLR6/MyD88/NF-κB signaling pathway, and promoting cell proliferation (Table1, Zhang *et al*., 2019).

MiRNAs Involved in Chicken Necrotic Enteritis

Necrotic enteritis (NE), commonly caused by *Clostridium perfringens*, is an acute clostridial disease that causes weight depression, loss of appetite, and sudden death (Hermans and Morgan, 2007). NE has re-emerged as a significant problem as a result of restrictions on the use of antibiotics in the poultry industry (Williams, 2005). In 2014, Dinh and colleagues investigated the effects of miRNAs on NE induced by *Eimeria maxima* and *Clostridium perfringens* in two genetically disparate chicken lines (Marek's disease resistant line 6_3 and Marek's disease-susceptible line 7_2). Their results demonstrated that miR-215, -217, -194, -200a, -200b, -216a, -216b, and -429 were highly expressed in intestine tissues derived from line 7_2 and that miR-1782 and -499 were down-regulated in these tissues. In spleen tissues, miR-34b and -1684 were the most up-regulated miRNAs in line 6_3. Additionally, the immune-related target genes (*CXCR5*, *BCL2*, *GJA1*, *TCF12* and *TAB3*) of these miRNAs were differentially expressed between the two chicken lines, and they were suppressed in the Marek's disease-susceptible chicken line (Dinh *et al*., 2014). Later, using the same chicken lines, Truong and colleagues explored the effects of TGF-β and miRNAs on NE. Members of the TGF-β family that is composed of TGF-β, BMPs, and SMADs function in the immune response (Escalona *et al*., 2012). According to their results, TGF-β1-3, BMP1-7, and SMAD1-9 were differentially expressed between the two chicken lines relative to their respective controls. Also, six miRNAs (gga-let-7c, -199, -200a, -200b, -429, and -499) were predicted and validated as the targets of *BMP7* (Truong *et al*., 2017a), indicating that the modulation of the TGF-β signaling pathway through BMP7 and miRNAs may play a role in the complex regulation of signaling pathways involved in the spleens of two NE-induced chicken lines. Additionally, the JAK-STAT signaling pathway is activated by over 40 cytokines and growth factors. This pathway is involved in cell differentiation, proliferation, apoptosis, and the progression of several diseases through its ability to regulate gene expression. In the spleens of chickens from line 6_3 and 7_2, 116 JAK-STAT pathway genes were found to be differentially expressed, and 63 mature miRNAs that variably target JAK-STAT pathway genes were also differentially expressed in the spleens of chickens from both lines (Truong *et al*., 2017b).

MiRNAs Involved in Chicken Ovarian Tumor

The laying hen is the only non-human animal that spontaneously develops ovarian cancer at a high prevalence rate, and this animal can be used as a model for ovary development and tumorigenesis (Johnson and Giles, 2013). Kang and colleagues found that in ovaries isolated from sexually mature (162-d) and sexually immature (42-d) chickens, 93 miRNAs were significantly differentially expressed, and these included gga-miR-1a, 21, 26a, 137, and 375 (Kang *et al*., 2013). Additionally, in recent years, miRNA has been found to regulate ovarian tumorigenesis. Avian beta-defensin (AvBD) proteins are members of beta-defensin subfamily and can protect against various microorganisms (van Dijk et al., 2008). In a study by Lim *et al*., gga-miR-1615 could influence *AvBD-11* expression via its 3'-UTR, and *AvBD-11* was most abundant in the glandular epithelium of endometrioid-type ovarian tumors, but not normal ovaries of laying hens, suggesting that *AvBD-11* and gga-miR-1615 may be useful as biomarkers for the diagnosis of ovarian cancer (Table 1, Lim *et al*., 2013).

Conclusions

Our knowledge regarding the functions of miRNAs in chickens has expanded in the past several decades. Current studies provide promising evidence concerning the ability of miRNAs to regulate immune function and diseases in chickens. Compared to the number of human studies, however, very few studies have been performed in farm animal to determine the mechanisms underlying the function of miRNAs. In these studies, many were limited to the identification of new and differentially expressed miRNAs or to the prediction of possible targets, and only a small number of these studies further validated the function of these miRNAs and the predicted targets experimentally. Recently, new mechanisms of miRNA action have been discovered, including the ability of these molecules to spread throughout the body when transported within exosomes and to act as protein ligands. Thus, continued mechanistic studies are required to more fully assess the impact of these miRNAs on the biological processes in chickens. Progress in the study of the roles and mechanisms of miRNAs in chickens will provide new ideas and targets for the improvement of chicken growth and the treatment of chicken diseases.

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References

Ahanda MLE, Ruby T, Wittzell H, Bed'Hom B, Chausse AM, Morin V, Oudin A, Chevalier C, Young JR and Zoorob R. Non-coding RNAs revealed during identification of genes involved in chicken immune responses. Immunogenetics, 61: 55–70. 2009.

Azad AA, Barrett SA and Fahey KJ. The characterization and molecular cloning of the double-stranded RNA genome of an Australian strain of infectious bursal disease virus. Virology, 143: 35–44. 1985.

Bannister SC, Smith CA, Roeszler KN, Doran TJ, Sinclair AH and Tizard MLV. Manipulation of estrogen synthesis alters MIR 202* expression in embryonic chicken gonads. Biology of Reproduction, 85: 22–30. 2011.

Bartel DP. MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell, 116: 281–297. 2004.

Bhoumik A, Gangi L and Ronai Z. Inhibition of melanoma growth and metastasis by ATF2-derived peptides. Cancer Research, 648222–8230. 2004.

Bhoumik A and Ronai Z. ATF2: a transcription factor that elicits oncogenic or tumor suppressor activities. Cell Cycle, 7: 2341–2345. 2008.

Biggs PM. The Leeuwenhoek Lecture. Marek's disease herpesvirus: oncogenesis and prevention. Philosophical Transactions of The Royal Society B-Biological Sciences, 352: 1951–1962. 1997.

Burnside J, Bernberg E, Anderson A, Lu C, Meyers BC, Green PJ, Jain N, Isaacs G and Morgan RW. Marek's disease virus encodes MicroRNAs that map to meq and the latency-associated transcript. Journal of Virology, 80: 8778–8786. 2006.

Burnside J, Ouyang M, Anderson A, Bernberg E, Lu C, Meyers BC, Green PJ, Jain N, Isaacs G and Morgan RW. Deep sequencing of chicken microRNAs. BMC Genomics, 9: 185. 2008.

Bushati N and Cohen SM. microRNA functions. Annual Review of Cell and Development Biology, 23: 175. 2007.

Carrington JC and Ambros V. Role of microRNAs in plant and animal development. Science, 301: 336–338. 2003.

Chen J, Wang Z, Bi D, Hou Y, Zhao Y, Sun J and Peng X. gga-miR-101-3p plays a key role in Mycoplasma gallisepticum (HS Strain) infection of chicken. International Journal of Molecular Sciences, 16: 28669–28682. 2015.

Chi J, Teng M, Yu Z, Xu H, Su J, Zhao P, Xing G, Liang H, Deng R, Qu L, Zhang G and Luo J. Marek's disease virus-encoded analog of microRNA-155 activates the oncogene c-Myc by targeting LTBP1 and suppressing the TGF-β signaling pathway. Virology, 476: 72–84. 2015.

Dang L, Teng M, Li H, Ma S, Lu Q, Hao H, Zhao D, Zhou E, Zhang G and Luo J. Marek's disease virus type 1 encoded analog of miR-155 promotes proliferation of chicken embryo fibroblast and DF-1 cells by targeting hnRNPAB. Veterinary Microbiology, 207: 210–218. 2017.

Dai Z, Ji J, Yan Y, Lin W, Li H, Chen F, Liu Y, Chen W, Bi Y and Xie Q. Role of gga-miR-221 and gga-miR-222 during tumour formation in chickens infected by subgroup J avian leukosis virus. Viruses, 7: 6538–6551. 2015.

Davidson W, Nettles V, Couvillion C and Yoder HW Jr. Infectious sinusitis in wild turkeys. Avian Diseases, 26: 402–405. 1982.

Denli AM, Tops BB, Plasterk RH, Ketting RF and Hannon GJ. Processing of primary microRNAs by the Microprocessor complex. Nature, 432: 231–235. 2004.

Di Cristofano A and Pandolfi PP. The multiple roles of PTEN in tumor suppression. Cell, 100: 387–390. 2000.

Dinh H, Hong YH and Lillehoj HS. Modulation of microRNAs in two genetically disparate chicken lines showing different necrotic enteritis disease susceptibility. Veterinary Immunology and Immunopathology, 159: 74–82. 2014.

Enright AJ, John B, Gaul U, Tuschl T, Sander C and Marks DS. MicroRNA targets in Drosophila. Genome Biology, 5: R1. 2003.

Escalona R, Diaz V, Pedernera E, Mendez C. Transforming growth factor beta mRNA and protein expression in the ovary of the chicken embryo. Growth Factors, 30: 297–303. 2012.

Friedman RC, Farh KK, Burge CB and Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Research, 19: 92–105. 2009.

Fu M, Wang B, Chen X, He Z, Wang Y, Li X, Cao H and Zheng S. gga-miR-130b suppresses infectious bursal disease virus replication via targeting the viral genome and cellular SOCS5. Journal of Virology, 92: e01646–17. 2017.

Fu M, Wang B, Chen X, He Z, Wang Y, Li X, Cao H and Zheng S. gga-miR-454 suppresses infectious bursal disease virus (IBDV) replication via directly targeting IBDV genomic segment B and cellular Suppressors of Cytokine Signaling 6 (SOCS6). Virus Research, 252: 29–40. 2018.

Gao Y, Yun B, Qin L, Pan W, Qu Y, Liu Z, Wang Y, Qi X, Gao H and Wang X. Molecular epidemiology of avian leukosis virus subgroup J in layer ocks in China. Journal of Clinical Microbiology, 50: 953–960. 2012.

Gigek CO, Lisboa LC, Leal MF, Silva PN, Lima EM, Khayat AS, Assumpcao PP, Burbano RR, Smith Mde A. SMARCA5 methylation and expression in gastric cancer. Cancer Investigation, 29: 162–166. 2011.

Grabarczyk, P, Przybylski gK, Depke M, Volker U, Bahr J, Assmus K, Broker BM, Walther R and Schmidt CA. Inhibition of BCL11B expression leads to apoptosis of malignant but not normal mature T cells. Oncogene, 26: 3797–3810. 2007.

Gutierrez A, Kentsis A, Sanda T, Holmfeldt L, Chen SC, Zhang J, Protopopov A, Chin L, Dahlberg SE, Neuberg DS, et al. The BCL11B tumor suppressor is mutated across the major molecular subtypes of T-cell acute lymphoblastic leukemia. Blood, 118: 4169–4173. 2011.

Han B, Lian L, Li X, Zhao C, Qu L, Liu C, Song J and Yang N. Chicken gga-miR-130a targets HOXA3 and MDFIC and inhibits Marek's disease lymphoma cell proliferation and migration. Molecular Biology Reports, 43: 667–676. 2016.

Harvey K and Tapon N. The Salvador-Warts-Hippo pathway - an emerging tumour-suppressor network. Nature Reviews Cancer, 7: 182–191. 2007.

Hermans PG and Morgan KL. Prevalence and associated risk factors of necrotic enteritis on broiler farms in the United Kingdom; a cross-sectional survey. Avian Pathology, 36: 43–51. 2007.

Hirai K and Shimakura S. Structure of infectious bursal disease virus. Journal of Virology, 14: 957–964. 1974.

Hicks JA, Tembhurne P and Liu HC. MicroRNA expression in chicken embryos. Poultry Science, 87: 2335–2343. 2008.

Hicks JA, Tembhurne PA and Liu HC. Identification of microRNA in the developing chick immune organs. Immunogenetics, 61: 231–240. 2009.

Hong S, Kim HY, Kim J, Ha HT, Kim YM, Bae E, Kim TH, Lee KC and Kim SJ. Smad7 protein induces interferon regulatory factor 1-dependent transcriptional activation of caspase 8 to restore tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis. Journal of Biological Chemistry, 288: 3560–3570. 2013.

Hu Q, Zhao Y, Wang Z, Hou Y, Bi D, Sun J and Peng X. Chicken gga-miR-19a targets ZMYND11 and plays an important role in host defense against Mycoplasma gallisepticum (HS Strain) infection. Frontiers in Cellular and Infection Microbiology, 6: 102. 2016.

Huguier S, Baguet J, Perez S, van Dam H and Castellazzi M. Transcription factor ATF2 cooperates with v-Jun to promote growth factor-independent proliferation in vitro and tumor formation in vivo. Molecular and Cellular Biology, 18: 7020–7029. 1998.

Huang BY, Hu P, Zhang DD, Jiang GM, Liu SY, Xu Y, Wu YF, Xia X and Wang Y. C-type natriuretic peptide suppresses mesangial proliferation and matrix expression via a MMPs/TIMPs-independent pathway in vitro. Journal of Receptor and Signal Transduction Research. 37: 355–364. 2017.

Ingle H, Kumar S, Raut AA, Mishara A, Kulkarni DD, Kameyama T, Takaoka A, Akira S and Kumar H. The microRNA miR-485 targets host and influenza virus transcripts to regulate antiviral immunity and restrict viral replication. Science Signaling, 8: ra126. 2015.

Jansson MD and Lund AH. MicroRNA and cancer. Molecular Oncology, 6: 590–610. 2012.

Johnson PA and Giles JR. The hen as a model of ovarian cancer. Nature Reviews Cancer, 13: 432–436. 2013.

Kang L, Cui X, Zhang Y, Yang C and Jiang Y. Identification of miRNAs associated with sexual maturity in chicken ovary by Illumina small RNA deep sequencing. BMC Genomics, 14: 352. 2013.

Keyomarsi K, O'Leary N, Molnar G, Lees E, Fingert HJ and Pardee AB. Cyclin E, a potential prognostic marker for breast cancer. Cancer Res, 54: 380–385. 1994.

Kibenge FS, McKenna PK and Dybing JK. Genome cloning and analysis of the large RNA segment (segment A) of a naturally avirulent serotype 2 infectious bursal disease virus. Virology, 184: 437–440. 1991.

Koparde P and Singh S. Avian influenza and micro RNA: Role of bioinformatics. Journal of Bioinformatcs and Sequence Analysis, 2: 12–22. 2011.

Kreager KS. Chicken industry strategies for control of tumor virus infections. Poultry Science, 77: 1213–1216. 1998.

Landoni MF and Albarellos G. The use of antimicrobial agents in broiler chickens. The Veterinary Journal, 205: 21–27. 2015.

Ley DH. Mycoplasma gallisepticum infection. Diseases of Poultry. 2008.

Levy A.M, Izumiya Y, Brunovskis P, Xia L, Parcells MS, Reddy SM, Lee L, Chen HW and Kung HJ. Characterization of the chromosomal binding sites and dimerization partners of the viral oncoprotein Meq in Marek's disease virus-transformed T cells, Journal of Virology, 77: 12841–12851. 2003.

Li Z, Zhang Y, Li Y, Zheng H, Zheng Y and Liu C. Distinct expression pattern of miRNAs in Marek's disease virus infected-chicken splenic tumors and non-tumorous spleen tissues. Research in Veterinary Science, 97: 156–161. 2014a.

Li X, Lian L, Zhang D, Qu L and Yang N. gga-miR-26a targets NEK6 and suppresses Marek's disease lymphoma cell proliferation. Poultry Science, 93: 1097–1105. 2014b.

Li H, Shang H, Shu D, Zhang H, Ji J, Sun B, Li H and Xie Q. gga-miR-375 plays a key role in tumorigenesis post subgroup J avian leukosis virus infection. PLoS ONE, 9 e90878. 2014c.

Li Z, Wang Y, Li X, Li X, Cao H, Zheng SJ. Critical roles of glucocorticoid-induced leucine zipper in infectious bursal disease virus (IBDV)-induced suppression of type I interferon expression and enhancement of IBDV growth in host cells via interaction with VP4. Journal of Virology, 87: 1221–1231. 2013.

Li H, Ji J, Xie Q, Shang H, Zhang H, Xin X, Chen F, Sun B, Xue C, Ma J, et al. Aberrant expression of liver microRNA in chickens infected with subgroup J avian leukosis virus. Virus Research, 169: 268–271. 2012.

Li Z, Chen B, Feng M, Ouyang H, Zheng M, Ye Q, Nie Q and Zhang X. MicroRNA-23b promotes avian leukosis virus subgroup J (ALV-J) replication by targeting IRF1. Scientific Reports, 5: 10294. 2015.

Li Z, Luo Q, Xu H, Zheng M, Abdalla BA, Feng M, Cai B, Zhang X, Nie Q and Zhang X. MiR-34b-5p suppresses melanoma differentiation-associated gene 5 (MDA5) signaling pathway to promote avian leukosis virus subgroup J (ALV-J)-infected cells proliferaction and ALV-J replication. Frontiers in Cellular Infection Microbiology, 7: 17. 2017.

Lian L, Li X, Zhao C, Han B, Qu L, Song J, Liu C and Yang N. Chicken gga-miR-181a targets MYBL1 and shows an inhibitory effect on proliferation of Marek's disease virus-transformed lymphoid cell line. Poultry Science, 94: 2616–2621. 2015.

Lin S and Gregory RI: MicroRNA biogenesis pathways in cancer. Nature Review Cancer, 15: 321. 2015.

Lim W, Jeong W, Kim J, Yoshimura Y, Bazer FW, Han JY and Song G. Expression and regulation of beta-defensin 11 in the oviduct in response to estrogen and in ovarian tumors of chickens. Molecular and Cellular Endocrinology, 366: 1–8. 2013.

Liu C, Zheng S, Wang Y, Jing L, Gao H, Gao Y, Qi X, Qin L, Pan W and Wang X. Detection and molecular characterization of recombinant avian leukosis viruses in commercial egg-type chickens in China. Avian Pathology 40: 269–275. 2011.

Liu D, Dai M, Zhang X, Cao W and Liao M. Subgroup J avian leukosis virus infection of chicken dendritic cells induces apoptosis via the aberrant expression of microRNAs. Scientific Reports, 6: 20188. 2016.

Luo W, Wu H, Ye Y, Li Z, Hao S, Kong L, Zheng X, Lin S, Nie Q and Zhang X. The transient expression of miR-203 and its inhibiting effects on skeletal muscle cell proliferation and differentiation. Cell Death & Disease, 5: e1347. 2014.

Luo J, Man T, Fan J, Wang F, Zhou L, Deng R and Zhang G. Marek' s disease virus-encoded microRNAs: genomics, expression and function. Science China-Life Sciences, 53: 1490–1490. 2010.

Magenta A, Greco S, Gaetano C and Martelli F: Oxidative stress and microRNAs in vascular diseases. International Journal of Molecular Science, 14: 17319–17346. 2013.

Mali AV, Joshi AA, Hegde MV and Kadam SS. Enterolactone suppresses proliferation, migration and metastasis of MDA-MB-231 breast cancer cells through inhibition of uPA induced plasmin activation and MMPs-mediated ECM remodeling. Asian Pacific Journal of Cancer Prevention, 18: 905–915. 2017.

Moon SK, Kim HM and Kim CH. PTEN induces G1 cell cycle arrest and inhibits MMP-9 expression via the regulation of NF-kappab and AP-1 in vascular smooth muscle cells. Archives of Biochemistry and Biophysics, 421: 267–276. 2004.

Muylkens B, Coupeau D, Dambrine G, Trapp S and Rasschaert D. Marek's disease virus microRNA designated Mdv1-pre-miR-M4 targets both cellular and viral genes. Archives of Virology, 155: 1823–1837. 2010.

Nicholas R and Ayling R. Mycoplasma in cattle. Veterinary Research, 178: 478–479. 2016.

Nath Neerukonda S, Egan NA, Patria J, Assakhi I, Tavlarides-Hontz P, Modla S, Munoz ER, Hudson MB and Parcells MS. Comparison of exosomes purified via ultracentrifugation (UC) and total exosome isolation (TEI) reagent from the serum of Marek's disease virus (MDV)-vaccinated and tumor-bearing chickens. Journal of Virological Methods, 263: 1–9. 2019.

Osterrieder N, Kamil JP, Schumacher D, Tischer BK and Trapp S. Marek's disease virus: from miasma to model. Nature Reviews Microbiology, 4: 283–294. 2006.

Ouyang W, Wang Y, Du X, Liu H and Zhang H. gga-miR-9* inhibits IFN production in antiviral innate immunity by targeting interferon regulatory factor 2 to promote IBDV replication. Veterinary Microbiology, 178: 41–49. 2015.

Ouyang W, Wang Y, Meng K, Pan Q, Wang X, Xia X, Zhu Y, Bi Z, Zhang H and Luo K. gga-miR-2127 downregulates the translation of chicken p53 and attenuates chp53-mediated innate immune response against IBDV infection. Veterinary Microbiology, 198: 34–42. 2017.

Ouyang W, Qian J, Pan Q, Wang J, Xia X, Wang X, Zhu Y and Wang Y. gga-miR-142-5p attenuates IRF7 signaling and promotes replication of IBDV by directly targeting the chMDA5's 3' untranslated region. Veterinary Microbiology, 221: 74–80. 2018.

Paul MC, Vergne T, Mulatti P, Tiensin T and Iglesias I. Editorial: Epidemiology of avian influenza viruses. Frontiers in Veterinary Science, 6: 150. 2019.

Peng X, Gao Q, Zhou L, Chen Z, Lu S, Huang H, Zhan C and Xiang M. MicroRNAs in avian influenza virus H9N2-infected and non-infected chicken embryo fibroblasts. Genetics and Molecular Research, 14: 9081–9091. 2015.

Pitcovski J, Gutter B, Gallili G, Goldway M, Perelman B, Gross G, Krispel S, Barbakov, M and Michael A. Development and large-scale use of recombinant VP2 vaccine for the prevention of infectious bursal disease of chickens. Vaccine, 21: 4736–4743. 2003.

Rathjen T, Pais H, Sweetman D, Moulton V, Munsterberg A and Dalmay T. High throughput sequencing of microRNAs in chicken somites. Febs Letters, 583: 1422–1426. 2009.

Saucedo LJ and Edgar BA. Filling out the Hippo pathway. Nature Reviews Molecular Cell Biology, 8: 613–621. 2007.

Schexnailder R and Griffith M. Liver fat and egg production of laying hens as influenced by choline and other nutrients. Poultry Science, 52: 1188–1194. 1973.

Shao F, Wang X, Yu J, Jiang H, Zhu B and Gu Z. Expression of miR-33 from an SREBF2 intron targets the FTO gene in the chicken. Plos One, 9: e91236. 2014.

Stipkovits L, Egyed L, Palfi V, Beres A, Pitlik E, Somogyi M, Szathmary S and Denes B. Effect of low-pathogenicity influenza virus H3N8 infection on Mycoplasma gallisepticum infection of chickens. Avian Pathology, 41: 51–57. 2012.

Stopka T, Zakova D, Fuchs O, Kubrova O, Blafkova J, Jelinek J, Necas E and Zivny J. Chromatin remodeling gene SMARCA5 is dysregulated in primitive hematopoietic cells of acute leukemia. Leukemia, 14: 1247–1252. 2000.

Tian F, Luo J, Zhang H, Chang S and Song J. MiRNA expression signatures induced by Marek's disease virus infection in chickens. Genomics, 99: 152–159. 2012.

Truong AD, Hong Y, Lee J, Lee K, Lillehoj HS and Hong YH. TGF-β signaling and miRNAs targeting for BMP7 in the spleen of two necrotic enteritis-afflicted chicken lines. Korean Journal of Poultry Science, 44: 211–223. 2017a.

Truong AD, Rengaraj D, Hong Y, Hong GT, Hong YH and Lillehoj HS. Differentially expressed JAK-STAT signaling pathway genes and target microRNAs in the spleen of necrotic enteritis-afflicted chicken lines. Research in Veterinary Science, 115: 235–243. 2017b.

Uddin MB, Ahmed SSU, Hassan MM, Khan SA and Mamun MA. Prevalence of poultry diseases at Narsingdi, Banglandesh. International Journal of Bioresearch, 1: 9–13. 2010.

van Dijk A, Veldhuizen EJ and Haagsman HP. Avian defensins. Veterinary Immunology and Immunopathology, 124: 1–18. 2008.

von Einem UI, Gorbalenya AE, Schirrmeier H, Behrens SE, Letzel T and Mundt E. VP1 of infectious bursal disease virus is an RNA-dependent RNA polymerase. Journal of General Virology, 85: 2221–2229. 2004.

Wang Y, Brahmakshatriya V, Lupiani B, Reddy SM, Sobiam B, Benham AL, Gunaratne P, Liu H, Trakooljul N, Ing N, et al. Integrated analysis of microRNA expression and mRNA transcriptome in lungs of avian influenza virus infected broilers. BMC Genomics, 13: 278. 2012.

Wang B, Fu M, Liu Y, Wang Y, Li X, Cao H and Zheng SJ. gga-miR-155 enhances type I interferon expression and suppresses infectious burse disease cirus replication via targeting SOCS1 and TANK. Frontiers in Cellular and Infection Microbiology, 8: 55. 2018.

Wang P, Hou J, Lin L, Wang C, Liu X, Li D, Ma F, Wang Z and Cao X. Inducible microRNA-155 feedback promotes type I IFN signaling in antiviral innate immunity by targeting suppressor of cytokine signaling 1. The Journal of Immunology, 185:

6226–6233. 2010.

Wang Y, Ouyang W, Pan Q, Wang X, Xia X, Bi Z, Wang Y and Wang X. Overexpression of microRNA gga-miR-21 in chicken fibroblasts suppresses replication of infectious bursal disease virus through inhibiting VP1 translation. Antivirus Research, 100: 196–201. 2013a.

Wang Q, Ji X, Gao Y, Qi X, Wang X, Wang Y, Qin L, Gao H and Wang X. Overexpression of microRNA gga-miR-1650 decreases the replication of avian leukosis virus subgroup J in infected cells. Journal of General Virology, 94: 2287–2296. 2013b.

Wang R, Zhang Y, Lu J, Xia B, Yang Z, Zhu X, Zhou X and Huang P. The highly pathogenic H5N1 influenza A virus down-regulated several cellular MicroRNAs which target viral genome. Journal of Cellular and Molecular Medicine, 21: 3076–3086. 2017.

Wang Y, Brahmakshatriya V, Zhu H, Lupiani B, Reddy SM, Yoon B, Gunaratne PH, Kim JH, Chen R, Wang J and Zhou H. Identification of differentially expressed miRNAs in chicken lung and trachea with avian influenza virus infection by a deep sequencing approach. BMC Genomics, 10: 512. 2009.

Webby RJ and Webster RG. Are we ready for pandemic influenza? Science, 302: 1519–1522. 2003.

Whelan RA, Doranalli K, Rinttila T, Vienola K, Jurgens G and Apajalahti J. The impact of Bacillus subtilis DSM 32315 on the pathology, performance, and intestinal microbiome of broiler chickens in a necrotic enteritis challenge. Poultry Science, pey500. 2018.

Williams RB. Intercurrent coccidiosis and necrotic enteritis of chickens: Rational, integrated disease management by maintenance of gut integrity. Avian Pathology, 34: 159–180. 2005.

Xu Z, Yu Y, Liu Y, Ou C, Zhang Y, Liu T, Wang Q and Ma J. Differential expression of pro-inflammatory and anti-inflammatory genes of layer chicken bursa after experimental infection with infectious bursal disease virus. Poultry Science, pez312. 2019.

Yoder HW Jr. Mycoplasma gallisepticum infection. Diseases of Poultry, 9: 198–212. 1991.

Yoo SJ, Huh JR, Muro I, Yu H, Wang L, Wang SL,Renny Feldman EM, Clem RJ, Muller HJ and Hay BA. Hid, Rpr and Grim negatively regulate DIAP1 levels through distinct mechanisms. Nature Cell Biology, 4: 416–424. 2002.

Yuan Bo, Zou M, Zhao Y, Zhang K, Sun Y and Peng X. Up-regulation of miR-130b-3p activates the PTEN/PI3K/AKT/NF-κB pathway to defense against Mycoplasma gallisepticum (HS strain) infection of chicken. International Journal of Molecular Sciences, 19: 2172. 2018.

Zambon MC. Epidemiology and pathogenesis of influenza. Journal of Antimicrobial Chemotherapy, 44: 3–9. 1999.

Zhao C, Li X, Han B, You Z, Qu L, Liu C, Song J, Lian L and Yang N. Gga-miR-219b targeting BCL11B suppresses proliferation, migration and invasion of Marek’s disease tumor cell MSB1. Scientific Reports, 7: 4247. 2017a.

Zhao Y, Hou Y, Zhang K, Yuan B and Peng X. Identification of differentially expressed miRNAs through high-throughput sequencing in the chicken lung in response to Mycoplasma gallisepticum HS. Comparative Biochemistry and Physiology-Part D, 22: 146–156. 2017b.

Zhao Y, Wang Z, Hou Y, Zhang K and Peng X. gga-miR-99a targets SMARCA5 to regulate Mycoplasma gallisepticum (HS strain) infection by depressing cell proliferation in chicken. Gene, 627: 239–247. 2017c.

Zhao Y, Yao Y, Xu H, Lambeth L, Smith LP, Kgosana L, Wang X and Nair V. A functional microRNA-155 ortholog encoded by the oncogenic Marek’s disease virus. Journal of Virology, 83: 489–492. 2009.

Zhao Y, Xu H, Yao Y, Smith LP, Lydia K, Green J, Petherbridge L, Baigent SJ and Nair V. Critical role of the virus-encoded microRNA-155 ortholog in the induction of Marek’s disease lymphomas. Plos Pathogens, 7: e1001305. 2011.

Zhang K, Han Y, Wang Z, Zhao Y, Fu Y and Peng X. gga-miR-146c Activates TLR6/MyD88/NF-κB Pathway through Targeting MMP16 to Prevent Mycoplasma Gallisepticum (HS Strain) Infection in Chickens. Cells, 8: 501. 2019.