Anticancer and immunomodulatory activity of egg proteins and peptides: a review

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ABSTRACT Eggs are widely recognized as a highly nutritious food source that offer specific health benefits for humans. Eggs contain all of the proteins, lipids, vitamins, minerals, and growth factors necessary for embryonic development. In particular, egg white and yolk proteins are considered functional food substances because they possess biological activities such as antimicrobial, antioxidant, metal-chelating, antihypertensive, anticancer, and immunomodulatory activities. Peptides produced via processes such as enzymatic hydrolysis, fermentation by microorganisms, and some chemical and physical treatments of egg proteins have been shown to enhance the functional properties and solubility of these peptides. Peptide activity is strongly related to amino acid sequence, composition, and length. At present, cancer remains among the leading causes of mortality worldwide, and therefore research aimed at developing new treatments for cancer immunotherapy is of great interest. The present review focuses primarily on the anticancer and immunomodulatory activities of egg proteins and their peptides and provides some insight into their underlying mechanisms of action. A number of egg proteins and peptides have been reported to induce apoptosis in cancer cells, protect against DNA damage, decrease the invasion ability of cancer cells, and exhibit cytotoxic and antimutagenic activity in various cancer cell lines. Furthermore, egg proteins and peptides can stimulate or suppress pro- or anti-inflammatory cytokines, as well as affect the production of inflammatory mediators in a variety of cell lines. In addition, the composition of eggs and the processes of egg proteins and peptides production will be discussed.

Key words: egg protein, egg peptide, functional activity, anticancer activity, immunomodulatory activity

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INTRODUCTION

Eggs are a highly nutritious food source that offer specific health benefits to humans. It is well known that eggs contain all of the nutrients necessary to maintain a developing embryo, including proteins, lipids, vitamins, minerals, and growth factors. In addition, eggs are an excellent source of protein of high biological value (Froning, 2006). A number of studies have shown that egg proteins possess many important biological activities including antioxidant, antimicrobial, and antihypertensive properties.

Today, the prevention and treatment of cancer as well as immunomodulation and immune stimulation are of great interest to the food and pharmaceutical industries. Cancer, characterized by uncontrolled cell proliferation and metastasis to many parts of the body, remains one of the leading causes of mortality worldwide, and cancer immunotherapy is an important aspect of cancer research and treatment (El-Gohary and Shaaban, 2017; Lohmueller and Finn, 2017). Therefore, bioactive materials that display anticancer and immunomodulatory activity are beginning to be studied. Ginseng is the most popular natural material in this field, and many reports have described its immunomodulatory activity (Hwang et al., 2019; Kang et al., 2019; Liu et al., 2019; Vinh et al., 2019; Yi, 2019). In addition to this, extracts from various plants (Ahmad et al., 2018; Alaklabi et al., 2018; Dong et al., 2018; Wang et al., 2018; Ma et al., 2019), compounds from marine products (Murugan and Iyer, 2014; Fernando et al., 2018; Yang et al., 2019b) and some probiotics (Haghshenas et al., 2015; Yang et al., 2019a; Zhao et al., 2019) are reported to have anticancer or immunomodulatory activities. In particular, proteins and peptides obtained from various sources—including soy (Kim et al., 2000; Kong et al., 2008), whey (Mercier et al., 2004; Saint-Sauveur et al., 2008; Attaallah et al., 2012), plant seed (Udenigwe et al., 2009; Liu et al., 2018), and fish proteins (Hsu et al., 2011; Hou et al., 2012; Halldorsdottir et al., 2014; Kim et al., 2018)—have been found to have anticancer and immunomodulatory activities.

Egg white and egg yolk are rich in proteins such as ovalbumin, ovotransferrin, ovomucin, ovomucoid, lysozyme, IgY, and phosvitin. Many studies have been undertaken to identify novel functional activities of egg

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proteins and to produce functional peptides from these proteins. In this review, the composition of eggs and the processes used to produce egg proteins and peptides have been discussed. Furthermore, their functional activities have been reviewed, with a specific focus on anticancer and immunomodulatory properties.

CHEMICAL COMPOSITION OF THE EGG

Egg Shell

Egg shell is composed of minerals, proteins, and water. Minerals are the major component (approximately 91%) and include compounds of calcium (98%), magnesium (0.9%), and phosphorus (0.9%) (Sugino et al., 1996). Egg shell consists of an outer layer (the cuticle) and an inner layer (the shell membrane). The cuticle layer is composed of proteins and a small amount of carbohydrates and lipids, and the shell membrane contains a small amount of protoporphyrin.

Egg White

Egg white accounts for approximately 60% of the total weight of the egg. Water and proteins are the major components of egg white, accounting for roughly 88 and 10.4%, respectively (Sugino et al., 1996). Carbohydrates, lipids, and minerals are minor constituents (less than 1%). Egg white consists of various proteins, of which ovalbumin is the most abundant (54%), followed by ovotransferrin (12%), ovomucoid (11%), ovoglobulin (4%), ovomucin (3.5%), lysozyme (3.4%), ovomacroglobulin (0.5%), and other, less abundant proteins. Egg white contains a trace amount of lipids (0.02%) (Sato et al., 1973) and includes minerals, such as compounds of sulfur, potassium, sodium, chlorine, phosphorus, calcium, magnesium, and iron. Carbohydrates exist in egg white in both a free (0.4% of egg white) and bound (0.5% of egg white) form. Glucose accounts for the majority of free carbohydrates (98%) in egg white (Sugino et al., 1996).

Egg Yolk

Egg yolk constitutes approximately 28% of the whole egg by weight, and the main component is lipids (60% by dry weight) (Sugino et al., 1996). Egg yolk lipids largely consist of triglycerides, phospholipids, cholesterol, and cerebroside. Most egg yolk proteins are in the form of low-density lipoprotein (**LDL**) and high-density lipoprotein (**HDL**). LDL accounts for 65% of total egg yolk protein and is responsible for its emulsifying properties. Phosvitin, livetin, and riboflavin-binding proteins are also present in egg yolk. The latter contains 1% minerals, the most abundant of which is phosphorus. More than 61% of egg yolk phosphorus is found in phospholipids. Carbohydrates account for 1% of egg yolk content and consist largely of oligosaccharides, which is composed of mannose and glucosamine (Sugino et al., 1996).

METHODS FOR SEPARATION OF EGG PROTEINS AND PREPARATION OF PEPTIDES

Various methods have been used to separate and purify proteins from egg white and egg yolk. Initially, salts such as ammonium sulfate, sodium chloride, or potassium chloride were used to separate ovalbumin (Warner and Weber, 1951), ovotransferrin (Fraenkel-Conrat and Feeney, 1950), ovomucin (Brooks and Hale, 1959), and phosvitin (Mecham and Olcott, 1949). However, the purity of these isolated proteins was very low. Chromatography methods are now commonly used to separate egg proteins. Ovalbumin (Sakakibara and Yanagisawa, 2007), lysozyme (Li-Chan et al., 1986), ovotransferrin (Croguennec et al., 2001), and phosvitin (Lei and Wu, 2012) were separated by ion exchange chromatography. Ovotransferrin (Al-Mashikhi and Nakai, 1987), IgY (Jiang et al., 2016), and lysozyme (Junowicz and Charm, 1975) can be separated by affinity chromatography. These chromatographic methods can yield high-purity proteins. Nonetheless, they are not suitable for large-scale production because of the high cost, the slowness of the process, and the low capacity. Ultrafiltration is a suitable method for the separation and purification of egg proteins. Many egg proteins such as ovalbumin (Datta et al., 2009), lysozyme (Wan et al., 2006), and IgY (Hernandez-Campos et al., 2010) can be separated by ultrafiltration. However, these ultrafiltration methods are highly complex and are strongly affected by operating and physicochemical conditions; therefore, despite the fact that they yield purity values greater than 90%, they cannot be scaled up.

Many peptides produced from egg proteins offer not only nutritional value but also bioactivities. These peptides often have no bioactivity in the native protein and must undergo proteolysis to acquire their specific bioactivities (Korhonen and Philanto, 2006). The most popular method for the production of functional peptides is enzymatic hydrolysis by means of commercial enzymes. Protease, trypsin, pepsin, α-chymotrypsin, alcalase, and papain are used for proteolysis (Mine et al., 2004; Pellegrini et al., 2004; Moon et al., 2013; Abeyrathne et al., 2016). These commercial enzymes are effective at producing peptides because their specific features, such as optimal pH, optimal temperature, and cleavage site, are well known. Additionally, some physical methods such as high pressure and heat treatments are employed for enzymatic hydrolysis to increase enzymatic digestibility (Hoppe et al., 2013; Ren et al., 2015). Non-commercial enzymes have also been used to prepare functional peptides from egg proteins (Eckert et al., 2014; Pokora et al., 2014), and those

Table 1. Biological activities of egg white and yolk proteins and the derived peptides.

	Protein	Biological activities	References
Egg white	Ovalbumin	Antioxidant activity	Huang et al. (2012)
		Antimicrobial activity	Pellegrini et al. (2004)
		Anticancer activity	Vis et al. (1998)
		Immunomodulatory activity	Fan et al. (2003); Goldberg et al. (2003); He et al. (2003); Rupa et al. (2015); Vidovic et al. (2002)
	Ovotransferrin	Antioxidant activity	Kim et al. (2012)
		Antihypertensive activity	Majumder and Wu (2010, 2011)
		Antimicrobial activity	Abdallah and Chahine (1999); Ibrahim et al. (2000); Moon et al. (2012); Valenti et al. (1983)
		Anticancer activity	Ibrahim and Kiyono (2009); Lee et al. (2017a); Moon et al. (2012, 2013)
		Immunomodulatory activity	Huang et al. (2010); Lee et al. (2018); Majumder et al. (2013); Xie et al. (2002)
	Lysozyme	Antihypertensive activity	Yoshii et al. (2001)
		Antimicrobial activity	Hughey and Johnson (1987); Ibrahim et al. (1991, 1992, 1994); Mine et al. (2004); Pellegrini et al. (2000)
		Anticancer activity	Das et al. (1992); Mahanta et al. (2015); Pacor et al. (1996, 1999); Sava (1989); Sava et al. (1989, 1991, 1995); Scherbakova et al. (2002)
		Immunomodulatory activity	Asakura et al. (1990); Ha et al. (2013); Sava (1996); Sugahara et al. (2000)
	Cystatin	Antimicrobial activity	Blankenvoorde et al. (1996)
		Anticancer activity	Cegnar et al. (2004); Muehlenweg et al. (2000); Premzl et al. (2001); Saleh et al. (2003)
		Immunomodulatory activity	Kato et al. (2000); Verdot et al. (1996, 1999)
	Avidin	Antimicrobial activity	Korpela et al. (1984)
		Anticancer activity	Corti et al. (1998); Gasparri et al. (1999); Moro et al. (1997)
	Ovomucin	Antimicrobial activity	Kobayashi et al. (2004)
		Anticancer activity	Oguro et al. (2000); Ohami et al. (1993); Watanabe et al. (1998)
		Immunomodulatory activity	Sun et al. (2016); Tanizaki et al. (1997)
Egg yolk	Phosvitin	Antioxidant activity	Katayama et al. (2006); Sakanaka et al. (2004); Xu et al. (2007)
		Metal chelating activity	Castellani et al. (2004)
		Antimicrobial activity	Khan et al. (2000); Ma et al. (2013)
		Anticancer activity	Moon et al. (2014)
		Immunomodulatory activity	Hu et al. (2013); Lee et al. (2017b); Ma et al. (2013); Xu et al. (2012)
	IgY	Anticancer activity	Amirijavid and Hashemi (2015); Amirijavid et al. (2014)
		Immunomodulatory activity	Li et al. (2016)
	Livetin	Immunomodulatory activity	Meram and Wu (2017)

derived from cheaper sources can reduce the cost of hydrolysis.

Purification techniques can also be used to increase the purity and functional activity of peptides. These techniques include low-molecular-weight cutoff membranes, gel filtration, ion exchange chromatography, and reverse-phase high-performance liquid chromatography (Rao et al., 2012a; Liu et al., 2015; Nimalaratne et al., 2015). Antioxidant and antihypertensive peptides with higher activity than that of crude hydrolysates have been obtained using these purification steps.

BIOLOGICAL ACTIVITIES OF EGG PROTEINS AND PEPTIDES

Various biological activities related to egg proteins and peptides have been reported and are summarized in Table 1.

Antioxidant Activity

Egg yolk phosvitin has a metal-chelating effect (Castellani et al., 2004), and as a result is a strong antioxidant. Furthermore, phosvitin oligophosphopeptides produced by tryptic hydrolysis of phosvitin demonstrate strong antioxidant activity in DPPH free-radical-scavenging assays and in Caco-2 cells (Katayama et al., 2006; Xu et al., 2007). Sakanaka et al. (2004) reported that egg yolk protein hydrolysates display antioxidant activities in a linoleic-acid oxidation system. One particular peptide resulting from the pepsin-mediated hydrolysis of crude egg white proteins was shown to have a strong antioxidant activity and high-radical-scavenging activity (Davalos et al., 2004). An ovalbumin–saccharide mixture has been reported to have stronger reducing power, greater DPPH-scavenging activity, and higher Trolox equivalent antioxidant capacity values than those of ovalbumin (Huang et al., 2012). Kim et al.

(2012) reported that ovotransferrin from egg white protein has antioxidant properties, and that this is further improved following hydrolysis by various enzymes.

Antihypertensive Activity

The 3 peptides (IRW, IQW, and LKP) derived from thermolysin- and pepsin-mediated ovotransferrin hydrolysis are capable of exerting a strong inhibitory effect on angiotensin-converting enzyme (**ACE**) (Majumder and Wu 2010, 2011). In addition, Rao et al. (2012b) reported that a hydrolysate of hen egg white lysozyme has potent ACE-inhibitory effects. Oligopeptides prepared from egg yolk protein were shown to suppress the development of hypertension in spontaneously hypertensive rats via inhibition of ACE activity (Yoshii et al., 2001).

Antimicrobial Activity

The bacteriolytic activity of lysozyme, which is often used as a natural food preservative (Hughey and Johnson, 1987), involves hydrolysis of the β(1-4) linkage between *N*-acetylmuramic acid and *N*-acetylglucosamine of peptidoglycan. It is typically most effective against gram-positive bacteria; however, chemical modification has been shown to increase its antimicrobial action against gram-negative bacteria (Ibrahim et al., 1991, 1992, 1994). Enzymatic hydrolysis of lysozyme has been found to enhance its antibacterial activity by producing peptides. More specifically, peptides corresponding to amino acid residues (aa) 15–21, 98–108 (Mine et al., 2004), and 98–112 (Pellegrini et al., 2000) were shown to have antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*.

Peptides resulting from enzymatic digestion of ovalbumin were found to be strongly active against *Bacillus subtilis* and to a lesser extent against gram-negative bacteria such as *E. coli*, *Bordetella bronchiseptica*, *Pseudomonas aeruginosa*, and *Serratia marcescens* (Pellegrini et al., 2004).

Ovotransferrin, a member of the transferrin family, can bind iron, and is thought to function as an iron scavenger, preventing iron utilization by microorganisms (Abdallah and Chahine, 1999). It has also been shown to have antibacterial activity against a wide range of bacteria, including *Pseudomonas* spp., *E. coli*, *Streptococcus mutans* (Valenti et al., 1983), *S. aureus*, *Bacillus cereus* (Abdallah and Chahine, 1999), *Listeria monocytogenes*, and *Helicobacter pylori* (Moon et al., 2012). Ovotransferrin antimicrobial peptide (OTAP)-92 consists of 92 aa located within the aa 109–200 region and has been shown to have antibacterial properties (Ibrahim et al., 2000). Under thermal stress, phosvitin from egg yolk exerts bactericidal action against *E. coli* and *S. aureus* owing to an increase in its metal-chelating ability and high surface activity (Khan et al., 2000; Ma et al., 2013). Furthermore, ovomucin (Kobayashi et al., 2004), cystatin (Blankenvoorde et al., 1996), avidin (Korpela et al., 1984), ovomacroglobulin (Miyagawa et al., 1991), and IgY (Kovacs-Nolan and Mine, 2004) have all demonstrated antimicrobial activities against several food poisoning–associated bacteria.

ANTICANCER ACTIVITIES OF EGG PROTEINS AND PEPTIDES

A number of studies have demonstrated the anticancer activities of egg proteins and associated peptides, and these can occur via a variety of mechanisms (Table 2).

Cystatin has been widely studied as an anticancer drug. It is known to inhibit the tumor-associated activity of intracellular cysteine proteases, possibly due to the presence of a urokinase-type plasminogen activator receptor binding site (Muehlenweg et al., 2000). Its ability to inhibit cysteine proteinases resulted in a reduction in tumor invasion ability and metastasis of Ras-transformed breast epithelial cells (Premzl et al., 2001). Cystatin has also been shown to be toxic toward MCF-10A neoT cells (Cegnar et al., 2004), and poly(lactide-co-glycolide) nanoparticles have been shown to improve the bioavailability and delivery of cystatin into tumor cells. Finally, Saleh et al. (2003) reported that purified egg white cystatin reduces the effects of cathepsins B and L, which have higher activity in gastric cancer tissues.

Avidin has been used in a wide variety of applications in the life sciences and in medicine, especially in pre-targeting of cancer treatments. Avidin has a strong affinity for biotin, and is highly thermostable and resistant to proteolysis by proteinase K (Hytonen et al., 2003). Moro et al. (1997) and Corti et al. (1998) studied the antitumor properties of avidin using avidin-biotinylated tumor necrosis factor-α (**TNF-α**). The addition of avidin was shown to increase the binding and persistence of TNF-α on tumor cells and increase the antitumor activity of TNF-α at least 5-fold, but importantly did not increase toxicity, as judged by assessing animal survival after treatment (Gasparri et al., 1999).

Lysozyme has been shown to have cancer-preventive properties when administered to normal mice (Das et al., 1992), and can directly activate immune cells and increase tumor cell immunogenicity (Sava et al., 1989). Lysozyme coupled with monomethoxypolyethylene glycol (**mPEG**-lysozyme) reduces tumor growth and prevents lung metastasis. However, this causes it to lose its enzymatic action against the *Micrococcus lysodeikticus* cell wall, meaning that the effect of lysozyme on tumor growth is unrelated to immunoactive peptidoglycan production (Pacor et al., 1996). mPEG-lysozyme has also been shown to reduce the number of tumor cells in the synthesis and pre-mitotic phases of the cell cycle (Pacor et al., 1999). A number of in vivo tests

Table 2. Egg proteins and peptides with anticancer activity.

Protein	Enzymes used for making peptides	Mechanism of activity	References
Cystatin	–	- Inhibited tumor-associated activity of intracellular cysteine protease - Reduced the activities of cathepsins B and L which are related with invasion ability of cancer cells	Cegnar et al. (2004) Muehlenweg et al. (2000) Premzl et al. (2001) Saleh et al. (2003)
Avidin	–	- Used in avidin-biotinylated TNF-α system and led to increased binding and persistence of TNF-α on tumor cells	Corti et al. (1998) Gasparri et al. (1999) Moro et al. (1997)
Lysozyme	–	- Directly activated immune cells or increased tumor cell immunogenicity - Reduced tumor growth and prevented tumor metastasis formation	Das et al. (1992) Mahanta et al. (2015) Pacor et al. (1996, 1999) Sava (1989) Sava et al. (1989, 1991, 1995) Scherbakova et al. (2002)
Phosvitin	–	- Inhibited the growth of cancer cell lines - Protected the damage of DNA	Moon et al. (2014)
IgY	–	- Recognized DR 5 receptor of TRAIL which induces apoptosis of MCF-7 cells	Amirijavid and Hashemi (2015) Amirijavid et al. (2014)
Ovomucin	Pronase	- β-subunit of ovomucin showed cytotoxicity in cultured tumor cells - Glycopeptide had an antitumor activity preventing edematous changes and neoangiogenesis of tumor cells	Oguro et al. (2000) Ohami et al. (1993) Watanabe et al. (1998)
Ovotransferrin	Trypsin, protamex, neutrase, flavozyme, α-chymotrypsin, alcalase, protex 6L, collupulin MG, thermolysin, promod 278	- Remarkably inhibited the proliferation of cancerous MCF-7 and HCT-116 cells - Induced apoptosis through mitochondrial pathway - Showed increased cytotoxic activity after enzyme hydrolysis - Two-step enzyme hydrolysates showed higher cytotoxic activity than single enzyme hydrolysates	Ibrahim and Kiyono (2009) Lee et al. (2017a) Moon et al. (2012, 2013)
Egg white protein	Alcalase, chymotrypsin, esperase, neutrase, pepsin, promod 278, trypsin	- Induced cell death by blocking G2/M phase in mouse lymphoma cell line	Yi et al. (2003)
Ovalbumin	–	- Heat-denatured ovalbumin exhibited antimutagenic activity against MNNG in *E. coli* DNA repair system	Vis et al. (1998)

TRAIL, TNF-related apoptosis-inducing ligand.

have suggested that lysozyme inhibits tumor growth and metastasis (Sava, 1989; Sava et al., 1991, 1995; Shcherbakova et al., 2002). Self-assembled nanostructured lysozyme (**snLYZ**) can be synthesized using a simple desolvation technique (Mahanta et al., 2015). It displays anticancer activities and has superior structural and functional stability at a wide range of pH and temperatures. Treating MCF-7 breast cancer cell lines with snLYZ resulted in 95% cell death, which was shown to occur via a reactive oxygen species-based mechanism (Mahanta et al., 2015).

Phosvitin has been shown to display cytotoxic activity against human cancer cell lines including HeLa (cervix), MCF-7 (breast), AGS (stomach), A549, SK-MES-1 (lungs), HepG2 (liver), and Hep-2 (larynx) cells (Moon et al., 2014), and does so in a dose-dependent manner. Furthermore, Moon et al. (2014) reported that phosvitin has protective effects against DNA damage in human leukocytes induced by H_2O_2 stimulation. DNA damage is well known to be related to cancer metabolism; thus, protecting against DNA damage is one way of preventing cancer.

IgY raised against a small 21 aa peptide from the human DR5 protein was shown to display anticancer activity against the MCF7 human cancer cell line (Amirijavid et al., 2014, 2015). IgY has high affinity for the DR5 protein, which is known to contribute to the overall apoptotic properties of TNF-related apoptosis-inducing ligand in cancer cells. Therefore, IgY induces death of MCF7 cells, but importantly does not appear to be toxic to normal cells (Amirijavid and Hashemi, 2015).

Hen egg white ovomucin consists of an α-subunit and a β-subunit. The β-subunit was reported to be toxic to tumor cells in culture (Ohami et al., 1993), and glycopeptides produced from pronase-treated ovomucin were shown to have antitumor activities in a double-grafted tumor system (Watanabe et al., 1998). Oguro et al. (2000) reported that a highly glycosylated peptide from pronase-treated ovomucin appeared to have an antitumor activity, and treating tumors with this peptide prevented edematous changes and neoangiogenesis.

Ovotransferrin from egg white can be autocleaved using a nonthiol reductant (TCEP). The resulting

peptide, reduced autocleaved OTF (rac-OTF), markedly inhibits the proliferation of HCT-116 colon cancer cells and MCF-7 breast cancer cells, but does not affect normal human mammary cells (Ibrahim and Kiyono, 2009). This study also showed that rac-OTF induced apoptosis associated with the mitochondrial pathway. Moon et al. (2012) demonstrated that ovotransferrin has cytotoxic activity against human cancer cell lines according to an MTT assay, and this activity was markedly increased following enzyme-mediated hydrolysis (Moon et al., 2013). Furthermore, Lee et al. (2017a) reported that 2-step enzyme hydrolysates of ovotransferrin displayed higher cytotoxic activity against human cancer cell lines compared to single enzyme hydrolysates.

In another study, egg white proteins were digested and extracted using various proteases and solvents to generate anticancer peptides (Yi et al., 2003). In particular, the acetone extract of alcalase hydrolysates displayed stronger antiproliferative activity against mouse lymphoma cells and induced cell death by blocking the G2–M transition.

Ovalbumin was found to have antimutagenic properties when heat denatured (Vis et al., 1998). Furthermore, this heat-denatured ovalbumin was shown to exert the strongest antimutagenicity action compared to other proteins, including bovine serum albumin and soy protein.

In summary, this review of the literature shows that a number of egg proteins and peptides can be used in cancer therapy. There are many examples of studies demonstrating the anticancer activity of egg proteins, and this occurs via a variety of mechanisms, including inducing apoptosis, protecting against DNA damage, decreasing the invasion ability of cancer cells, and increasing the cytotoxic activity and antimutagenic activity of the cells. More in vivo testing and human studies will be needed before a new anticancer agent can be developed. To date, with the exception of lysozyme, most of the studies on egg proteins were conducted in vitro. Furthermore, current anticancer drugs have a number of problems, including toxicity to normal cells and high cost. Egg proteins and peptides can affect cell viability; therefore, toxicity tests on normal cells will be necessary in order to add to existing anticancer drugs. However, toxicity testing has been done in only a few studies, and most of these lack an investigation into the underlying molecular mechanism. There have been a number of reports on cell signaling pathways associated with cancer (e.g., PI3K/AKT/mTOR pathway, p53 pathway, NF-κB pathway, MAPK pathway). It will therefore be necessary to study the signaling pathways involved in the effect of egg proteins and peptides on cancer cells. Future studies on the anticancer activities of egg proteins and peptides are expected to include in vivo cytotoxic testing, followed by human studies, with the hope of revealing the molecular mechanisms and cell signaling pathways that are involved.

IMMUNOMODULATORY ACTIVITIES OF EGG PROTEINS AND PEPTIDES

There have been many reports describing the immunomodulatory activities of egg proteins and the derived peptides, which occur via a variety of mechanisms (Table 3).

Ovalbumin from egg white has been shown to stimulate TNF-α secretion from RAW 264.7 macrophages, when modified with methylglyoxal (Fan et al., 2003). Furthermore, Rupa et al. (2015) reported that heat-denatured ovalbumin affects the production of cytokines in $CD4^+$ T cells. Heat-denatured ovalbumin can also increase the production of interleukin (**IL**)-12, IL-17, and IL-10 and downregulate IL-4. Peptides produced from ovalbumin (corresponding to regions aa 77–84 and aa 126–134) cause an increase in macrophage phagocytic activity (Mine and D'Silva, 2008). Ovalbumin peptides have also been reported to enhance immune responses in cancer immune therapy (Vidovic et al., 2002; Goldberg et al., 2003; He et al., 2003).

The immunomodulatory properties of ovotransferrin were demonstrated by its ability to stimulate IL-6, nitrite, and metalloproteinase (MMP) production in HD 11 chicken macrophage cells (Xie et al., 2002), while Lee et al. (2018) showed that it can stimulate the production of proinflammatory cytokines in murine macrophages via the MAPK signaling pathway. IL-6 is a well-known contributor to adaptive immune responses, where it can activate T cells, increase B-cell proliferation, and upregulate antibody secretion during chronic inflammation. Huang et al. (2010) reported that the ovotransferrin-derived peptide IRW confers anti-inflammatory activities by inhibiting the production of intercellular adhesion molecule 1 (**ICAM-1**), vascular cell adhesion molecule 1 (**VCAM-1**), and monocyte chemotactic protein 1 (**MCP-1**) in endothelial cells when stimulated by TNF-α. IRW can also suppress p50 and p65 translocation, both of which are related to the NF-κB pathway (Majumder et al., 2013).

An ovomucin peptide produced by the alcalase enzyme was found to have anti-inflammatory activity, which occurs via inhibition of the TNF-mediated NF-κB pathway (Sun et al., 2016). Of the 3 ovomucin hydrolysates identified in this study, only the desalted alcalase hydrolysate displayed any anti-inflammatory activity, and more specifically was shown to suppress the TNF-induced expression of ICAM-1, a proinflammatory gene. Finally, Tanizaki et al. (1997) reported that ovomucin glycopeptides have a strong macrophage-stimulating activity, which occurs via an increase in H_2O_2 generation and IL-1 production.

Cystatin from egg white is known to upregulate nitric oxide release from interferon (**IFN**)-γ-activated macrophages (Verdot et al., 1996), and this upregulation originates from the synthesis of cytokines, such as TNF-α and IL-10 (Verdot et al., 1999). Cystatin has also been shown to affect IL-6 and IL-8 production in gingival fibroblasts (Kato et al., 2000).

Table 3. Egg proteins and peptides with immunomodulatory activity.

Protein	Enzymes used for making peptides	Mechanism of activity	References
Ovalbumin	Pepsin, chymotrypsin	- Stimulated TNF-α secretion - Heat-denatured ovalbumin affected the production of cytokines - Increased production of IL-12, IL-17, and IL-10 and decreased IL-4 - Peptides (OA 77–84, OA 126–134) increased in macrophage phagocytic activity	Fan et al. (2003) Goldberg et al. (2003) He et al. (2003) Rupa et al. (2015) Vidovic et al. (2002)
Ovotransferrin	Thermolysin, pepsin	- Stimulated the production of IL-6, nitrite, and MMP - Increased the production of proinflammatory cytokines via the MAPK pathway - IRW inhibited TNF-α induced production of ICAM-1, VCAM-1, and MCP-1 in endothelial cells - IRW suppressed the translocation of p50 and p65 (related to the NF-κB pathway)	Huang et al. (2010) Lee et al. (2018) Majumder et al. (2013) Xie et al. (2002)
Ovomucin	Alcalase, pronase-papain	- Inhibited TNF-mediated NF-κB pathway - Suppressed TNF-induced ICAM-1 expression - Stimulated macrophage activity by increasing H_2O_2 generation and IL-1 production	Sun et al. (2016) Tanizaki et al. (1997)
Cystatin	–	- Stimulated NO production in mouse peritoneal macrophages - Induced the synthesis of TNF-α and IL-10 - Upregulated IL-6 and IL-8 production in gingival fibroblasts	Kato et al. (2000) Verdot et al. (1996, 1999)
Lysozyme	–	- Stimulated production of immunoglobulin - Enhanced translation of IgM - Improved chronic sinusitis and bronchitis - Lysozyme-galactomannan conjugate (LGC) increased pro-inflammatory cytokine production via JNK, ERK, and NF-κB pathway	Asakura et al. (1990) Ha et al. (2013) Sava (1996) Sugahara et al. (2000)
Livetin	Pepsin, alcalase	- Suppressed the production of pro-inflammatory cytokines	Meram and Wu (2017)
IgY	–	- Attenuated the increase in IFN-γ and TNF-α and the decrease in IL-10 in *Salmonella* Typhimurium infected mice model	Li et al. (2016)
Phosvitin	Trypsin	- Reduced LPS-induced TNF-α release from murine RAW 264.7 cells - In the absence of LPS stimulation, increased production of pro-inflammatory cytokine and phagocytic activity of macrophages - Inhibited TNF-α and LPS stimulated expression of IL-8, IL-12, and MCP-1 in HT-29 cells	Hu et al. (2013) Lee et al. (2017b) Ma et al. (2013) Xu et al. (2012)

Sugahara et al. (2000) demonstrated that egg white lysozyme stimulates immunoglobulin production. Lysozyme enhances IgM synthesis and was shown to effectively improve chronic sinusitis and bronchitis (Asakura et al., 1990; Sava, 1996). Ha et al. (2013) studied the immunomodulatory activities of Maillard-type lysozyme-galactomannan conjugate, and found that it was capable of inducing nitric oxide and stimulating TNF-α, IL-1β, and IL-8 expression in macrophages. The same study determined that the immunomodulatory activities of lysozyme are due to stimulation of JNK, ERK, and NF-κB pathways.

Livetin from egg yolk was found to have anti-inflammatory activity in that it suppresses the production of proinflammatory cytokines such as TNF-α, IL-1β, IL-6, and IL-10 in LPS-induced RAW 264.7 macrophages (Meram and Wu, 2017). The enzymatic hydrolysates of livetin produced by pepsin and alcalase display strong anti-inflammatory activity. Livetin and its enzymatic hydrolysates decrease the production of nitric oxide and prostaglandin E2 (**PGE2**), which are strongly associated with various inflammatory diseases in which inducible nitric oxide (**iNOS**) and cyclooxygenase 2 (**COX-2**) are suppressed.

Egg yolk-derived IgY has been shown to display some immunomodulatory activity in the intestinal mucosa of mice infected with *Salmonella* Typhimurium (Li et al., 2016). Infection with *S.* Typhimurium caused an increase in the expression levels of proinflammatory cytokines such as TNF-α and IFN-γ, but after treatment with IgY, the mice showed downregulation of these cytokines, and their survival rate increased from 40% (untreated) to 80% (IgY treated).

Phosvitin from egg yolk has been reported to have some endotoxin-neutralizing effects in RAW 264.7 murine macrophages (Ma et al., 2013). LPS-induced TNF-α release was significantly inhibited in these cells following treatment with phosvitin. However, in the

absence of LPS stimulation, phosvitin was found to exhibit some immune-enhancing activity, via an increase in proinflammatory mediators (e.g., nitric oxide, iNOS, TNF-α, and IL-1β) and increased phagocytic activity of macrophages (Lee et al., 2017b). Hu et al. (2013) isolated peptides from phosvitin and examined their endotoxin-neutralizing capacity. They reported that phosvitin-derived peptide (Pt5e) was able to reduce TNF-α and IL-1β expression in RAW 264.7 macrophages and in mice. Phosphopeptides from phosvitin exert an anti-inflammatory activity on intestinal epithelial cells by downregulating key proinflammatory markers, such as TNF-α, IL-8, IL-12, and MCP-1 (Xu et al., 2012).

In summary, a number of studies have shown that egg proteins and peptides are good candidates for use as immunomodulatory agents in the food and pharmacological industries. These proteins and peptides are able to stimulate or suppress pro- or anti-inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-8, IL-10, and IL-12), and can affect the production of inflammatory mediators (nitric oxide, PGE2, iNOS, COX-2, ICAM-1, VCAM-1, and MCP-1) in a variety of cell lines. However, all of these studies have several limitations. First, the majority were conducted in vitro, rather than in an animal model or human study. This is important because the gastrointestinal environment contains substances such as bile salt, gastric acid, and a variety of proteases, all of which can disrupt the structure of egg proteins and peptides, and thus reduce their immunomodulatory activity. Studies that utilize animal models are therefore essential in order to test the stability and activity of functional proteins and peptides in the gastrointestinal environment before they could be used in industry. Second, there is a significant lack of studies addressing the cytotoxicity of egg proteins and peptides. Before functional proteins and peptides can be used in industry, it is essential to examine their cytotoxic activities, using animal models and human studies in addition to in vitro cell lines. Only after the safety of these functional proteins and peptides has been confirmed can the next step can be carried out. Finally, only a few studies have actually identified the functional peptides. Functional activity is related to peptide structure, amino acid sequence, and peptide length, and therefore identifying these features will be helpful in fully understanding their functional activity. In conclusion, future studies on the immunomodulatory activity of egg proteins and peptides should be accompanied by toxicity and efficacy assessments using animal models and human studies, and should be followed by purification and identification of the peptides.

CONCLUSION

Studying the functional activities of egg proteins and peptides is important for boosting the egg industry. Egg consumption, either as whole or processed eggs, has only a limited ability to stimulate the industry. However, as a result of studies into the functional properties of egg proteins and peptides, eggs can now serve not only as a functional food material, but also in the preparation of pharmaceutical materials. Such studies have concluded that peptides produced from egg proteins possess higher functional activities compared to the undigested protein. Peptide production from proteins is important for improving their functional activity and for increasing their absorption in the digestive tract. Nonetheless, there has been a lack of research into the reasons for the functional activity of peptides, in terms of their purification and characterization. There is therefore a great need for more studies into the purification and characterization of functional peptides that may have anticancer and immunomodulatory activities. Furthermore, many of the studies on egg proteins and peptides were conducted on cell lines in vitro. In order to use these functional materials in industry, more in vivo studies and/or human studies will be required to confirm their safety, activity, and stability in the gastrointestinal environment. Finally, current studies on the anticancer and immunomodulatory activities are focused on the gut microbiome, which has been shown to be strongly associated with human diseases, including cancer. Therefore, combining future studies on the anticancer and immunomodulatory activities of egg proteins and peptides with microbiome studies will be a very interesting and valuable area of research.

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