Subject Review

Zinc α2-Glycoprotein: A Multidisciplinary Protein

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Abstract

Zinc α2-glycoprotein (ZAG) is a protein of interest because of its ability to play many important functions in the human body, including fertilization and lipid mobilization. After the discovery of this molecule, during the last 5 decades, various studies have been documented on its structure and functions, but still, it is considered as a protein with an unknown function. Its expression is regulated by glucocorticoids. Due to its high sequence homology with lipid-mobilizing factor and high expression in cancer cachexia, it is considered as a novel adipokine. On the other hand, structural organization and fold is similar to MHC class I antigen-presenting molecule; hence, ZAG may have a role in the expression of the immune response. The function of ZAG under physiologic and cancerous conditions remains mysterious but is considered as a tumor biomarker for various carcinomas. There are several unrelated functions that are attributed to ZAG, such as RNase activity, regulation of melanin production, hindering tumor proliferation, and transport of nephritic by-products. This article deals with the discussion of the major aspects of ZAG from its gene structure to function and metabolism. (Mol Cancer Res 2008;6(6):892–906)

Introduction

Zinc α2-glycoprotein (ZAG) is a 40-kDa single-chain polypeptide (1), which is secreted in various body fluids (2). ZAG is known to stimulate lipolysis in murine epididymal adipocytes through stimulation of adenylate cyclase in a GTP-dependent process (3) via binding through β_3-adrenoreceptor (4). It is involved preferentially in depletion of fatty acids from adipose tissues, subsequently named as lipid-mobilizing factor (5). This factor, which is highly expressed in cancer cachexia (6), is characterized by the extensive reduction of fat in the human body. The protein assay (7) and mRNA expression (8) in the mammary tumor have shown that there is a relation between the ZAG levels at histologic grade of the breast cancer tumors. Moreover, many studies suggested that ZAG is also a potential serum marker of prostate cancer that may be elevated early in tumor growth (9).

High degree of similarity between ZAG and class I MHC molecules has been evaluated both at sequence and structural levels (10-12). The crystal structure of ZAG consists of a large groove analogous to class I MHC peptide-binding grooves. The structure and environment of groove reflect its role in immunoregulation and in lipid catabolism (11). The alteration in residues of the peptide-binding groove clearly showed uniqueness of ZAG among MHC class I–like proteins (13, 14). These observations indicate that ZAG might be able to bind with different peptides, antigens, and ligands. Hence, it was suggested that function of ZAG has diverged from the peptide presentation and T-cell interaction functions of class I MHC molecules (15).

There are various reports on different aspects of ZAG. The works, however, have never been reviewed. Here, we have compiled for the first time a detailed analysis of all the relevant information for ZAG that may be helpful in understanding of functional importance of ZAG in the body system.

Site of Expression

The ZAG was first reported in human serum and subsequently purified (1). The presence of ZAG in human seminal fluid was reported in 6-fold molar excess compared with human serum (16, 17), which suggested as a key element for fertilization without any experimental evidences. The serum ZAG is synthesized by the genes of liver. The seminal ZAG, however, is of prostatic origin (9, 16, 17). The presence of ZAG in normal human body fluids and kidney extract has been described (18). Jirka and Blanicky (19) have reported three isoforms of ZAG through immunoelectrophoresis and reported that concentration of each human serum ZAG isoform increases from its lowest value in the fetal and early newborn period to the highest ones in children and adults. Through immunohistochemical analysis, Mazoujian (20) studied normal skin and 41 benign sweat gland tumors and found that ZAG was expressed predominantly in tumors of apocrine differentiation. It was also, however, expressed in some tumors of eccrine differentiation (21). Abundant proteins expressed in human saliva are ZAG with some other proteins determined by proteomic analysis and mass fingerprinting. Recently, the gene expression of ZAG in normal human epidermal and buccal epithelia was reported (22). ZAG is also produced by adipocytes, where the mRNA of ZAG was detected by reverse transcription-PCR in the mouse

Received 12/10/07; revised 1/17/08; accepted 1/23/08.
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doi:10.1158/1541-7786.MCR-07-2195

white adipose tissue and in the interscapular brown fat. Finally, ZAG is synthesized by epithelial cells of prostate gland and liver, secreted into various body fluids such as serum (1), semen (23), sweat (24), saliva (24), cerebrospinal fluid (24), milk (24), urine (25), and amniotic fluid (26). The concentration of ZAG has been reported to increase dramatically in carcinomas (3). Therefore, it is also considered as a good biomarker for prostate (27), breast (28), oral (22), and epidermal (29) carcinomas.

Gene Structure and Regulation

The gene for ZAG, assigned to the chromosome 7q22.1 through fluorescent hybridization karyotyping, comprised four exons and three introns (30). The first exon (exon 1) is for the region from the cap site to the 6th amino acid (Gly), the second exon (exon 2) is for the region from the 6th to the 93rd amino acid (Gly), the third exon (exon 3) is for the region from the 93rd to the 185th amino acid (Asp), and the fourth exon (exon 4) is for the region from the 185th amino acid to the end of the mRNA (31). The exon 4 also contains the entire 3′-untranslated region, including a hexanucleotide AATAAA that probably represents the polyadenylation signal of the gene (32). The serum ZAG shows complete nucleotide sequence homology with that from the prostate, which includes the signal peptide (29). The full gene of ZAG was reported by Freije et al. (8). The gene sequence of ZAG includes an open reading frame encoding an 18-amino acid long hydrophobic signal peptide and the 278 residues of the mature protein. A comparison of the amino acid sequence deduced from the nucleotide sequence with that determined for the protein isolated from the serum through chemical methods (10) reveals some minor differences. There are two substitutions (i.e., Gln and Glu) present at positions 65 and 222 of the mature polypeptide chain, respectively, instead of the Glu and Gln residues at these positions as determined by the protein sequencing. In addition, the nucleotide sequencing of ZAG (8, 32) detected the insertion of an Ile-Phe pair between residues located at positions 75 and 76 of the previous sequence determined by chemical methods (10). The nucleotide sequence analysis of *ZAG* gene reported that there are some intervening sequences (32), and the length of intron is larger due to the presence of an unusually high density of Alu repetitive sequences within them. A total of nine Alu sequences were identified. The five are present in the first intron, and the remaining four in the second intron. This reflects that repetitive sequences are clearly overrepresented in the *ZAG* gene. Eight of these Alu sequences are oriented in the opposite direction of the ZAG, whereas the remaining one (Alu-6) is oriented in the same direction (32). In addition, two MER sequences belonging to subfamilies 12 and 14 (33) and one MIR element (34) were found in the first and second introns of the gene, respectively.

The gene expression of ZAG is predominantly regulated by androgens and progestins (35, 36). Glucocorticoids are also responsible for the increased ZAG expression in adipose tissue (37). Russell and Tisdale (38) examined its 5′-flanking region that could affect the transcription of the gene. They proposed that 5′-flanking region of the gene containing several consensus sequences could be relevant in the transcription of the *ZAG* gene. Finally, they suggested that ZAG expression is likely to be mediated by the interaction of several transcription factors acting synergistically on different *cis*-acting elements. In addition, the lipolytic action of dexamethasone was attenuated by anti-ZAG antibody, suggesting that the induction of lipolysis was mediated through an increase in ZAG expression. It was further proposed that expression of ZAG is mediated through the β_3-adrenoreceptor present on the *ZAG* gene (35).

ZAG as a Biomarker

Although the exact mechanism by which ZAG actively participates in tumor proliferation is not known, a large body of data exists in favor of the expression of ZAG with respect to the stages of tumor (39-42). ZAG is designated as a potential biomarker of different types of carcinomas (6, 7, 27, 35, 38, 43-46). ZAG is synthesized in the prostate itself, and its high concentrations in prostatic tissue and prostatic secretion should facilitate its action in prostate and in other tissues (9). Furthermore, the increased concentration of ZAG in semen is directly linked with the prostate pathophysiology. The immunohistochemical analysis revealed an elevated concentration of ZAG in prostatic adenocarcinoma (47). In other analysis, it was observed that the majority of prostate cancer cells tested (i.e., 35 of 48 cells) have reacted with anti-ZAG antibodies (44). Moreover, high-grade tumors expressed a minimal ZAG than the moderate-grade tumors (44). Recently, the gene expression of ZAG in prostate cancer was analyzed using cDNA microarrays (48). Interestingly, ZAG seemed to be one of the genes differentially expressed in prostate cancer samples. Furthermore, they evaluated ZAG expression through immunohistochemistry using a set of 232 tumors and found strong staining for ZAG, which was associated with a decreased risk of recurrence. The clinical and pathologic multivariate analyses were done by Hull et al. (49). They found four independent variables, pathologic stage, Gleason grade in the prostatectomy specimen, and surgical margin status. More recently, the expression of ZAG in a wide range of prostate cancer samples was determined for its role as a putative biomarker for prostate cancer progression using a semiautomated microscope system (50). There is a marked direct relation between ZAG expression and the tumor stage in prostate cancer. The ZAG expression has also been used to predict clinical recurrence and metastatic tumor progression. Low ZAG expression is associated with the clinical recurrence (27). We have also identified the elevated expression of ZAG in prostate cancer seminal fluid compared with the normal sample through two-dimensional gel electrophoresis and matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (51).

Apart from prostate cancer, ZAG is also considered as a potential biomarker for breast carcinoma due to its elevated concentration in breast tissues compared with that in the normal, benign, and malignant breast specimens (24). Many studies have confirmed that ZAG is a reliable immunohistochemical marker of apocrine cell differentiation of mammary epithelial tissues (7, 8, 35, 36, 52-56). Sanchez et al. (28) have shown that many proteins are expressed in breast cancer cell line, and ZAG is one of them. Interestingly, there is a higher ZAG level in the well-differentiated tumors than that in the moderately or poorly differentiated tumors. It was proposed that

ZAG may be considered as a biochemical marker of differentiation in breast cancer (7). Lopez-Otin and Diamandis (57) reviewed five common markers of prostate and breast cancer and ZAG is one of them.

ZAG is normally expressed in liver, but an increased expression of the gene has been reported in both benign and malignant breast tissues (53). This may raise the plasma concentration above the threshold required to produce lipolysis. The high-level expression of ZAG in cancer cachexia has been evaluated for many years (4-6, 37, 38, 45, 58). The ability of ZAG to stimulate lipolysis and cyclic AMP (cAMP) formation was compared with the effect of lipid mobilization factor (LMF) isolated from the cancer patient's urine. A direct relationship has been established in the expression of ZAG in cancer cachexia and their concentration in urine, and hence, subsequently, ZAG is also termed as a biomarker for cancer cachexia.

Apart from the above-mentioned carcinomas, ZAG is also considered as a potential tool for the investigation of other tumors. Recently, the biomarkers for liver fibrosis in hepatitis C patients were determined by using two-dimensional gel electrophoresis and mass spectrometry. The most prominent differences were observed when serum samples from cirrhotic patients were compared with those from a healthy control serum. Inter-α-trypsin inhibitor heavy chain H4 fragments, α1 antichymotrypsin, apolipoprotein L1, prealbumin, albumin, paraoxonase/arylesterase 1, and ZAG were decreased in cirrhotic serum, suggesting the role of ZAG there (39). Irmak et al. (41) used the same techniques followed by immunoblotting and immunohistochemistry and they identified two proteins, orosomucoid and human ZAG, which were increased in the urine samples of patients with bladder cancer in comparison with the urine samples of healthy volunteers. The gene expression of ZAG from histopathologically graded oral squamous cell carcinomas was compared with that in the perilesional normal. It was observed that ZAG levels are (*a*) higher in the controls than those in the tumors and (*b*) higher in well-differentiated tumors than those in the poorly differentiated tumors. These findings led to the conclusion that ZAG can also be considered as a marker of the oral epithelial maturation (59). Mazoujian (60) studied the immunohistochemical localization of ZAG in the normal skin and in 41 benign sweat gland tumors, and they found that ZAG was expressed predominantly in tumors of apocrine differentiation. It was also, however, expressed in some tumors of eccrine differentiation (21, 60). Recently, Abdul-Rahman et al. (43) for the first time determined the presence of ZAG in gynecologic cancer. They determined the higher expression of ZAG in serum of patients with squamous cell cervical carcinoma and cervical adenocarcinoma when compared with normal. In another experiments, García-Ramírez et al. (61) did the proteomic analysis of the human vitreous fluid by differential gel electrophoresis for an accurate quantitative comparison between patients with the proliferative diabetic retinopathy and nondiabetic human. They found that ZAG as a new potential candidate was involved in the pathogenesis of proliferative diabetic retinopathy. In other experiment, Jain et al. (25) have shown that ZAG is among the additional proteins in urine samples of microalbuminuria-positive diabetes patients. These proteins can be used as markers for specific and accurate clinical analyses of diabetic nephropathy. The peptide fingerprint of urine protein glomerular disease was analyzed, and they found that ZAG is a biomarker for glomerular disease (40). In conclusion, ZAG not only is a biomarker for prostate and breast cancer but also covers the most commonly occurring cancer.

ZAG and Cancer

The seminal plasma ZAG is synthesized by prostate epithelial cells and secreted into seminal fluid (62), and it constitutes 30% of the proteins present in the seminal fluid (18). Hale et al. (44) determined the expression of ZAG in prostate tumors and found that ZAG is produced by 73% of prostate cancers. Moreover, ZAG production is associated with tumor differentiation status. It is decreased or absent in more poorly differentiated tumors. They have also shown that this observation is similar to that of breast cancer, where the loss of ZAG production was associated with the lack of tumor differentiation. It is interesting to note that the expression of ZAG in prostate cancer is similar to that of other relevant prostatic proteins (57), such as prostate-specific antigen and prostatic acid phosphatase, which are significantly decreased in prostatic tumors (63-65). Moreover, immunohistochemical studies assured the partial loss of ZAG expression in prostatic tissue after malignant transformation (47). Recently, Lapointe et al. (48) determined the gene expression profiling of prostate cancer from 62 primary prostate tumors, as well as 41 normal prostate specimens and 9 lymph node metastases, using cDNA microarrays containing 26,000 genes. They suggested that prostate tumors can be usefully classified according to their gene expression patterns, and these tumor subtypes may provide a basis for improved prognostication and treatment stratification.

ZAG is a reliable immunohistochemical marker of apocrine cell differentiation in human breast (24) and standard prognostic factors in women with early breast cancer. Women with more advanced breast cancer had higher serum ZAG levels than those with the early stage of the disease. A comparative analysis in mammary tissues from women with different diseases revealed enhanced expression of *ZAG* gene in benign breast lesions and a variable expression level in breast cancers (7, 8). ZAG was expressed predominantly in tumors of apocrine and eccrine differentiations (60). ZAG is considered as a marker of oral epithelia (59). In a patient with cancer cachexia and prostate cancer together, ZAG production by prostate cancer can lead to systemically elevated serum ZAG levels that may be useful diagnostically (44).

Clinically, cachexia manifests with excessive weight loss in the setting of ongoing disease, usually with disproportionate muscle wasting (66). Many pieces of evidence suggest that cytokines play a central role in the pathogenesis of cachexia (67). The role of ZAG in cancer cachexia was determined by Bing et al. (6). They showed that ZAG is produced by both white and brown adipose tissues, and their mRNA and protein levels are markedly increased in adipose tissue of mice with cancer cachexia. Moreover, they suggested that ZAG may be a unique protein factor in the local modulation of lipid metabolism and contribute particularly to the substantial reduction of adipose in cancer cachexia. The ability of ZAG to induce uncoupling protein expression has been determined, and it has been found that ZAG directly influences the

expression of uncoupling proteins, which may play an important role in the lipid use during cancer cachexia (58). High sequence homology of ZAG with LMF further suggests the potential role of ZAG in the lipid mobilization (3, 45, 68). Despite these small chemical differences between ZAG and LMF, the biological activity of ZAG seems to be very similar to that of LMF, and pharmacologic and biochemical evidences indicate that both molecules induce lipolysis *in vitro* by a cAMP-mediated system through interaction with a β_3-adrenoreceptor (69). Russell et al. (4) used the freeze/thawing methods to determine the effect of ZAG on lipolysis *in vivo*. The alteration in the secondary structure due to freeze thaw alters the conformation of ZAG, which is important for the interaction of β_3-adrenoreceptor and may require ZAG for biological activity. Recently, Rolli et al. (12) used the ZAG-deficient mice to elucidate the pathophysiology of ZAG. They ablated both ZAG alleles in the mouse genome through standard gene-targeting technology and found that ZAG knockout mice were overweight with respect to wild-type littermates. That is, ZAG overexpression should lead to weight loss and eventually cachexia.

ZAG may be related to the development of superficial bladder cancer and to its switch to an invasive phenotype (41). In conclusion, ZAG is a clinically important protein, which is directly involved in various types of tumor proliferation.

Sequence Analysis

The first full polypeptide sequence of ZAG was determined by Araki et al. (10) through biochemical analysis followed by cDNA sequence determination of this protein from different sources by many groups (32, 70, 71). The studies suggested that all the ZAG from different sources has same polypeptide sequence. Some differences, however, occur at the posttranslational level. ZAG is synthesized as a 295-amino acid-long immature polypeptide, which, after deletion of NH_2-terminal 17-amino acid-long signal polypeptide, is converted into mature ZAG. Interestingly, the 18th residue (i.e., first of the mature ZAG) of the immature ZAG is glutamine, which is cyclized to form pyroglutamine. Hence, the serum ZAG has blocked NH_2 terminus. On the other hand, with the deletion of four to six NH_2-terminal residues of the mature ZAG, seminal ZAG has free NH_2 terminus. Recently, our group has purified a novel ZAG, which is complexed with the prolactin-inducible protein in the human seminal fluid and has blocked NH_2 terminus due to the presence of pyroglutamine.[4] The polypeptide structure of the mature ZAG comprised 278 amino acid residues. The amino acid composition, which has a molecular mass of 31,889 Da, is as follows: Asp = 18, Asn = 11, Thr = 9, Ser = l8, Glu = 21, Gln = 20, Pro = l7, Gly = l6, Ala = 18, Cys = 4, Val = 21, Met = 3, 11e = 9, Leu = 19, Tyr = 18, Phe = 9, Lys = 20, His = 7, Ag = 12, and Trp = 8. The presence of high tryptophan and tyrosine residues accounts for the high extinction coefficient ($E^{1\%}_{1cm}$) of 18.0. The 278-amino acid-long polypeptide folds into three domains; each is composed of ~90 amino acid residues, named as α1, α2, and α3 domains. The mature ZAG contains four putative glycosylation sites (Asn-Xaa-Ser/Thr) at Asn^{89}, Asn^{92}, Asn^{106}, and Asn^{239}. Of the four (except Asn^{92}), three Asn residues carry glucosamines. Interestingly, Asn^{239}, which is located on α3 domain, is the bulkiest, sialylated (90%) on the mannose at 2,6-arm. The three glycans possessed the typical N-linked biantennary structure, being substituted with *N*-acetylneuraminic acid in a 2,6-linkage and lacking fucose. The molecular mass of the carbohydrate moiety of ZAG is 6,644 Da and that of the native glycoprotein is 38,478 Da (10).

The amino acid sequence of ZAG contains four half-cystine residues. The position of cysteine and disulfide pairing determined through both biochemical and structural analysis reveals the bond formation in between residues 101 and 164 and second one between residues 203 and 258. Interestingly, the loops formed by the disulfide bonds are similar in length, 64 and 56 residues, respectively.

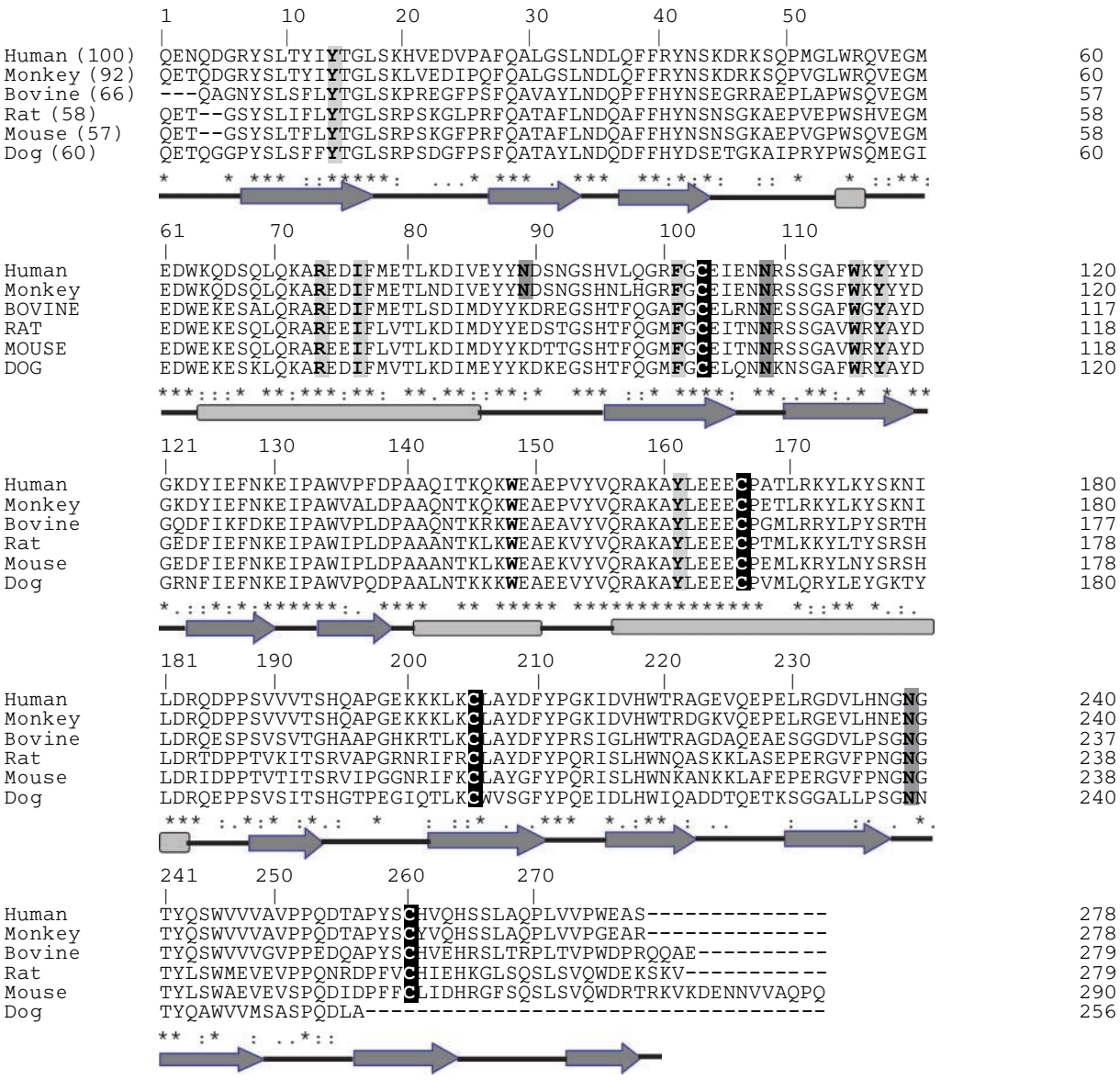
ZAG share high sequence homology with antigen-presenting molecules such as MHC-I, which will be discussed in the next section. The sequence comparison of the human ZAG with other mammalian ZAG molecules, which is available from genome projects, is illustrated in Table 1. The sequence alignment in the table clearly indicates regions of proteins that are highly conserved across the mammals and those that are divergent. Interestingly, the amino acids of α1-α2 domains are highly conserved, strongly indicative of the functional importance of the groove in the biological functions of ZAG that are evident from the crystal structure and ligand-binding mutational studies (13, 72). On the other hand, the α3 domain of ZAG is less conserved indicative of species specificity and evolutionary constrains. The four cysteine residues, which are involved in disulfide pairing, are conserved in all mammalian ZAG. Interestingly, of four putative glycosylation sites, two (Asn^{106} and Asn^{239}) are totally conserved in all the mammalian ZAG.

Fold and Stability

Secondary structure of ZAG, predicted from its sequence, revealed the presence of 23% α-helix, 27% β-sheet, and 22% β-turns (10). The polypeptide folds into three domains with almost equal number of amino acid residues. Both the α1 and α2 domains of ZAG have disulfide bonds in homologous positions to MHC-I, responsible for the antigen binding in case of MHC-I molecules. ZAG is considered as a member of immunoglobulin gene superfamily based on its amino acid sequence and domain structure (10). Interestingly, ZAG does not require peptides and β2-microglobulin (β_2M) for proper folding as MHC-I. The structure of classic MHC-I molecules is simply the assembly of the heavy chain with β_2M to form noncovalent heterodimer loaded with peptides that are usually 8 to 9 amino acids in length. These peptides were generated by cleavage of proteins in the cytosol. These peptides are actively transported by a heterodimeric complex, known as transporter associated with antigen presentation, into the endoplasmic reticulum, where assembly with class I molecules takes place (73). The trimeric complex is then transported through the Golgi apparatus to the cell surface. The crucial role of β_2M in the proper folding and function of MHC-I was determined using cell lines and knockout mice devoid of β_2M expression (74-77). These experiments led to conclude that β_2M (*a*) stabilizes cell surface expression of class I heavy chains, (*b*)

[4] M.I. Hassan et al., unpublished result.

TABLE 1. Multiple Sequence Alignment of Human ZAG with Other Mammalian ZAGs



NOTE: Swiss-Prot entry for *Homo sapiens* (human), P25311; *Macaca mulatta* (monkey), XP_001098813; *Bos taurus* (bovine), Q3ZCH5; *Rattus norvegicus* (rat), Q63678; *Mus musculus* (mouse), Q64726; and *Canis familiaris* (dog), Q4GX49. The conserved cysteine residues involved in disulfide formation are shaded in black. The residues shaded in light gray are involved in ligand binding. Asn residues shaded in dark gray are glycosylation sites. The sequence identities with human ZAG are written in parenthesis. The secondary structure elements are shown below the sequence as loop in black line, β strand as in arrow, and α-helix as a cylinder.

facilitates the binding of purified class I molecules to antigenic peptides both on cells and plastic surfaces, and (*iii*) generates additional high-affinity peptide-binding sites in preparations of soluble purified class I molecules (74-76).

The far-UV CD spectrum of the native ZAG measured at pH 7.4 and 25°C (77) shows an intense negative peak at 218 nm. The best fit for the spectrum between 210 and 250 nm was found with a linear combination of 3% α-helix, 60% β-sheet, and 1% β-turns. The remaining 36% represented unordered structure (random coil), which agrees with the earlier determined secondary structure from amino acid sequence (10). In the near-UV range (250-320 nm), positive CD peak was observed at 265 nm, most probably corresponding to the disulfide chromophores (77). Furthermore, a positive peak at 295 nm is typical of tryptophan residues. The reversible thermal transition curve was determined by observing changes in the CD signal at 218 nm, which disappeared gradually on heating the protein sample in the temperature range 25°C to 85°C. The midpoint of denaturation (T_m) estimated from this curve was 66°C. The classic MHC-I heavy chain is thermally less stable in the absence of either peptide (78-82) or β_2M (83), whereas ZAG exists as an isolated class I MHC-like heavy chain without bound peptides. The melting curve of ZAG shows a T_m of 65°C, which is comparable with previously obtained T_ms for

peptide-filled class I MHC molecules: H-2K^d, 57°C (78); HLA-A2, 66°C (84). The T_m (65°C) obtained from the ZAG melting curve is substantially higher than that (43-45°C) of an empty class I MHC molecule; H-2K^d complexed with human or murine β_2M (78, 85). Interestingly, the newly discovered complex of ZAG with prolactin-inducible protein by our group has shown dramatic increase in T_m, which is 75°C.[4] ZAG shows high stability in the absence of peptide and β_2M, which has been explained after solving the crystal structure of serum ZAG (11). The crystal structure of ZAG reveals that a network of hydrogen bonds is formed between α3 and α1-α2, whereas only single interaction has been made in β_2M-binding class I proteins. In addition, the extra hydrogen bonds between loop connecting β_4 strand (residues 51-54) and the helical region of the ZAG α1 domain platform α3 (236-241) are closer in ZAG compared with their MHC-I counterpart. Moreover, the burial of larger surface area 970 Å^2 in ZAG, which in class I molecules is 660 Å^2, leads to constraints in the position of the ZAG α3 domain relative to α1-α2 (11).

Structure of ZAG

The first crystal structure of human ZAG at 2.8 Å through X-ray crystallography was reported by Sanchez et al. in 1999 (11). After that, the structures of many mutants have been determined by the same group. The overall fold of ZAG is very similar to that of other classic MHC-I–like molecules, both in fold and quaternary structure arrangement, despite a low sequence similarity (see Fig. 1; refs. 11, 86-97). Like classic MHC-I molecules, the α1-α2 superdomains of ZAG adopt a fold that is symmetrical to immunoglobulin constant domains (93, 98-101). Recently, we have determined the crystal structure of human seminal ZAG complexed with prolactin-inducible protein, which shows same structural fold with very little root mean squares deviations for Cα atoms with that of serum ZAG.[4] The spatial arrangement of three domains (α1-α2 and α3) in ZAG lacking binding to β_2M is slightly different from that in MHC-I.

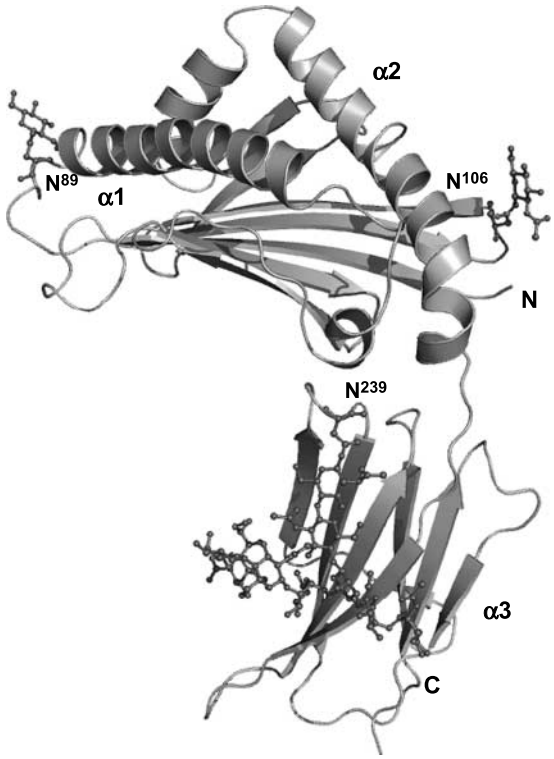


FIGURE 1. The ribbon diagram of ZAG representing three domains (α1, α2, and α3). The glycan chains are represented in ball and stick model.

The α1-α2 domain has been the focus of most interests because this part of the MHC-I structure is responsible for peptide binding and MHC-restricted recognition by T-cell receptor (TCR). The two domains share a common tertiary structure, composed of a four-stranded antiparallel β-pleated sheet that forms the NH_2-terminal half of the domain followed by a long α-helical region. The α-helices lie on top of the sheet and run antiparallel to each other forming a groove-like structure. The first helix in α1 is a short helix (residues 50-55) that meets a longer helix (residues 57-85). The helical region of α2 domain also starts with a short helix (residues 138-148) that meets a longer helix (residues 150-175). The large cleft between the α1-α2 helices is the most likely location of the single peptide-binding site proposed based on peptide competition experiments (21). In addition to the obvious fact, the cleft is on top of the molecule where it would be easily accessible to TCRs. The presence of polyethylene glycol, a hydrophobic ligand in the same groove surrounded by hydrophobic side chains along with the Arg^{73}, is indicative of the binding capability of ZAG with peptides and amphipathic molecules such as fatty acids (Fig. 2). This hydrophobic groove is a characteristic of ZAG and has its possible role in fatty acid depletion (8, 72) and regulation of lipid metabolism (3). Due to structural similarity with classic MHC-I molecules, such as CD1, hemochromatosis factor (HFE), and neonatal Fc receptor (FcRn), ZAG is also expected to bind with a variety of ligands *in vivo*. The different mutants of ZAG were crystallized and their fluorescence studies were done, which elucidated that Arg^{73} has an unexpectedly central role in the structure of the binding site (13). The substitution of Arg^{73} with several amino acid residues resulted in the apparent closure of the binding site, as is the case for other members of the MHC class I protein family (14). Furthermore, it was speculated that Arg^{73} has additional role of acting as an anchor for the ligand by interacting with the negatively charged head group of, for instance, a fatty acid. This is supported by substitution of Arg with Lys, which has the same effect via binding through different ligands (13).

The α3 domain primarily consists of seven antiparallel β-sheets. Although the serum ZAG is quite similar to seminal ZAG at a sequence level, extensive carbohydrate moiety is present at Asn^{239} minor glycan chains, which were observed at Asn^{89} and Asn^{106}. The fourth site Asn^{92}, however, is totally devoid of glycan chain. An earlier report on biochemical characterization of seminal ZAG describes the absence of glycan chain (17), but presently, we observed glycan chain at Asn^{239}.[4] The presence of glycan chain on α3 domains proposed its functional implication in cell signaling and binding capability with different cell surface receptors due to the presence of RGD sequence (Arg^{231}-Gly^{232}-Asp^{233}) at β_4 strand (102). This further supports its role in binding to cell surface receptor via integrin-mediated process. The α3 domains

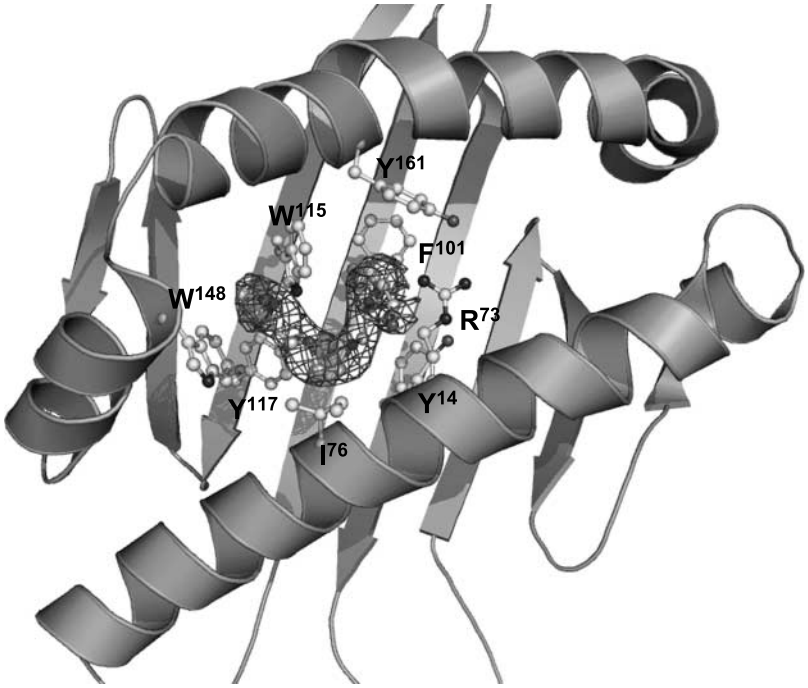


FIGURE 2. The α1-α2 domains form a groove, which is predominated by hydrophobic residues represented by ball stick model. Electron density for polyethylene glycol, which occupied this groove, is shown in mesh.

of human ZAG are only carrying RGD motif but the mouse or rat does not. The difference in RGD sequences of disintegrin and ZAG α3 domain is that it is present in the β strand instead of in loop of disintegrin. The earlier report that ZAG down-regulates cdc2, the only cyclin-dependent kinase regulating the G_2-M transition without redundancy, is required as a rate-limiting step in the cell cycle (103). An increased expression of ZAG has been directly linked to the increased proliferation and decreased differentiation of advanced tumors (59). Similarly, the down-regulation of ZAG might hinder tumor progression and can be directly related to the presence of glycan chain and RGDV motif. The importance of RGD motif on ZAG has also been determined in Tu-138 oral squamous cell carcinoma cell line where the proliferation is inhibited by the synthetic RGD peptides, such as RGD, RGDV, RGDS, and the ZAG matrix, compared with other matrix proteins, such as fibronectin, vitronectin, laminin, and collagens I and IV (102).

Functions of ZAG

After the discovery of ZAG in 1961 (1), many researchers are still working on ZAG. Even now, it is elucidated as a protein of unknown function. Its presence in human seminal plasma at high concentrations, however, postulated its role in fertility. A high degree of similarity with classic MHC-I molecules suggests its role in immunomodulation. Due to its presence and different degree of expression in various tissues and body fluids, several functions may be attributed to ZAG, which are described here.

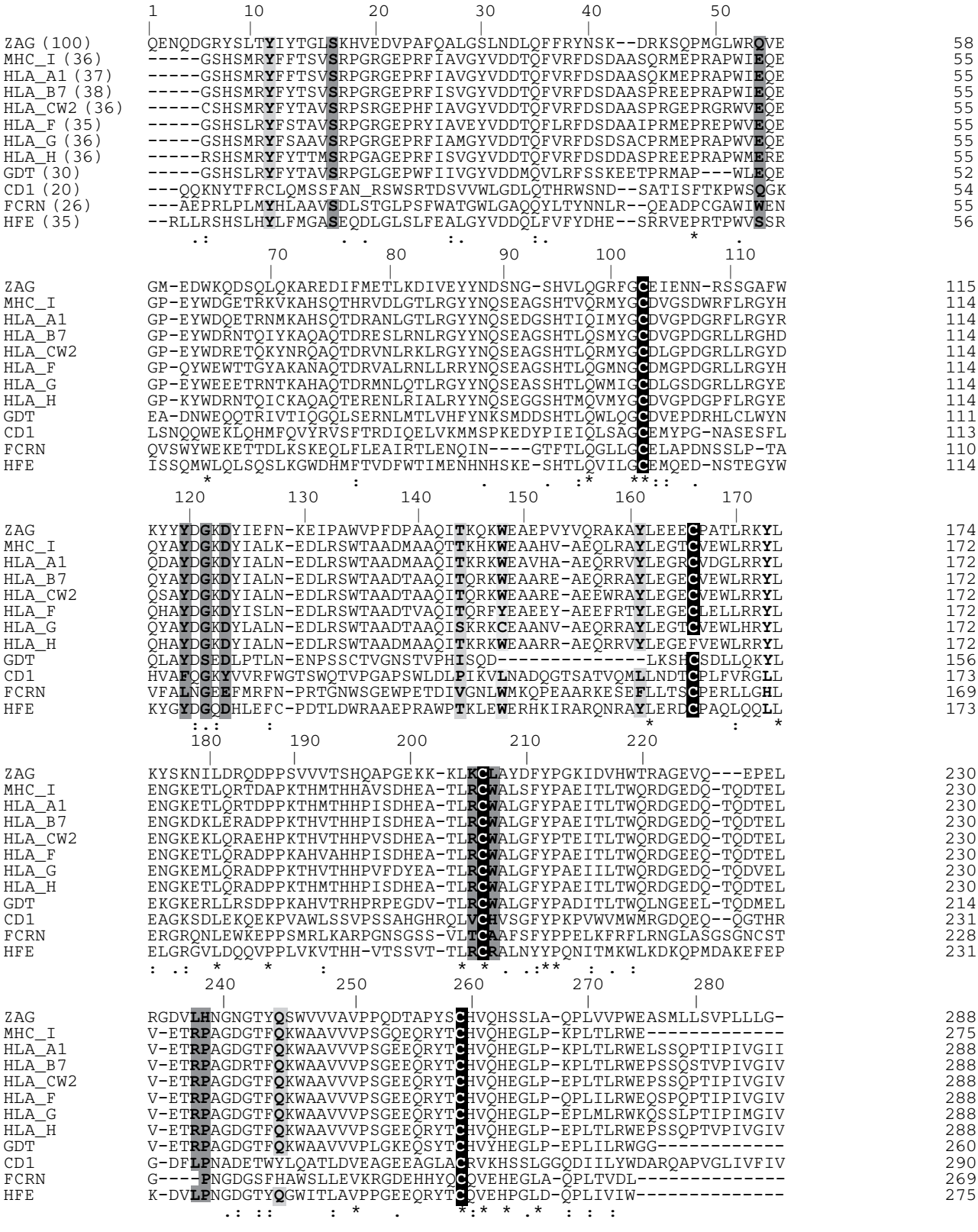
Fertilization

There is no evidence for the direct role of ZAG in fertility, although a high concentration of ZAG in human semen (6-fold to serum) suggested its role in fertility (17, 61). The sperm motility is a key factor to determine semen quality and its fertilizing capacity (104-106), which is widely controlled by a variety of factors (107-109). The phospholipid composition and cholesterol to phospholipid ratio are predominant factors required for the sperm maturation in the epididymis (110). The lipid metabolism pathways are presumably a significant mechanism for modulating sperm motility (111), and due to its lipid-metabolizing capability, ZAG may be predicted to play a vital role in such a process. ZAG is able to bind to the sperm membrane and initiate forward motility, which is directly linked to cAMP levels in semen. The mechanisms by which cAMP activates sperm cytosolic cAMP, which in turn phosphorylates multiple intra-sperm phosphoproteins that may regulate flagellar motility (112, 113) of spermatozoa. Moreover, ZAG is also a protein that is directly related to sperm motility and in turn fertilization (62).

As a Carrier Protein

The presence of glycoprotein excreted in the urine was thought to be derived after the damage of glomerular basement membrane. In the course of investigation on the glycoprotein isolated from the urine of a diseased group affected by glomerular basement membrane damage, it seemed, however, that a second source of the nephritogenic glycoprotein excreted in the urine is in blood plasma, which is ZAG. The common determinant with nephritogenic urinary glycoprotein and serum is ZAG, and finally, it was concluded that the plasma ZAG may play the role of a carrier protein of the nephritogenic renal glycoprotein (114). Due to deletion of COOH-terminal residues, ZAG acts as a soluble MHC-I molecule, which leads to the transport of nonpolymorphic substances or in intercellular recognition processes (32). The crystallographic studies of ZAG about the importance of its α1-α2 domain suggest that groove can bind a small hydrophobic ligand (13, 73). ZAG has been proposed to regulate lipid metabolism (3), and this function may be modulated by the binding of lipid-like

TABLE 2. Multiple Sequence Alignment of ZAG with Classic MHC-I–like Proteins



(*Continued on the following page*)

TABLE 2. Multiple Sequence Alignment of ZAG with Classic MHC-I–like Proteins (Cont'd)

ZAG	----------	
MHC_I	----------	
HLA_A1	AGLVLLGAV-ITGAVVAAVMWRRKSSDRKGGSYTQAASSDSAQGSDVSL-----	336
HLA_B7	AGLAVLAVV-VIGAVVAAVMCRRKSSGGKGGSYSQAACSDSAQGSDVSLTA---	338
HLA_CW2	AGLAVLAVLAVLGAVVAVVMCRRKSSGGKGGSCSQAASSNSAQGSDESLIASKA	342
HLA_F	AGLVVLGAV-VTGAVVAAVMWRKKSSDRNRGSYSQAAVTDSAQGSGVSLTANKV	341
HLA_G	AGLVVLAAV-VTGAAVAAVLWRKKSSD----------	314
HLA_H	AGLVLLVAV-VTGAVVAAVMWRKKSSDRKGGSYSQAASSNSAQGSDVSLTA---	338
GDT	----------	
CD1	LIMLVVVGAVVYYIWRRRSAYQDIR----------	315
FCRN	----------	
HFE	----------	

NOTE: Conserved cysteine residues involved in disulfide linkage are shaded in black. The conserved residues predicted for peptide binding are highlighted in light gray and residues involved in β_2M binding are dark gray. The sequences are as follows: ZAG, human serum ZAG (PDB: 1ZAG); MHC, class I histocompatibility antigen, HLA-A 0201 (PDB: 2CLR); HLA_A1, class I histocompatibility antigen, A-1 α chain from *Homo sapiens* (P30443); HLA_B7, HLA class I histocompatibility antigen, B-7 α chain from *Homo sapiens* (P01889); HLA_Cw2, HLA class I histocompatibility antigen, Cw-2 α chain from *Homo sapiens* (P30501); HLA_F, HLA class I histocompatibility antigen, α chain F from *Homo sapiens* (P30511); HLA_G, HLA class I histocompatibility antigen, α chain G from *Homo sapiens* (P17693); HLA_H, HLA class I histocompatibility antigen, α chain H from *Homo sapiens* (P01893); GDT, γ/δ TCR ligand (PDB: 1C16); CD1, CD1 (mouse) antigen-presenting molecule (PDB: 1CD1); FcRn (PDB: 3FRU); and HFE: HFE (human) hemochromatosis protein (PDB: 1A6Z).

molecules in the ZAG groove. As a soluble molecule, ZAG is likely to degrade and eliminate RNA by-products after processing within cells, as well as incorrectly synthesized or damaged RNAs (115). Various studies to identify molecule(s) that binds to ZAG *in vivo* will be required to elucidate the significance of the hydrophobic ligand-binding properties of the ZAG groove and to understand the function of ZAG in the binding and transport of various ligands.

Immunoregulation

High degree of sequence similarity and structural folds like MHC-I molecules, ZAG may be considered as a member of immunoglobulin superfamily, which seems to be a truncated secretory MHC-I–like protein. ZAG may have a role in the expression of the immune response (10, 11, 87-98). The members of this family typified by the class I MHC proteins, which present peptides to cytotoxic T cells (99), include CD1 (which presents lipidic antigens to T cells; refs. 100, 116), FcRn (which is involved in transportation of immunoglobulin across epithelia; ref. 95), and HFE (a transferring receptor-binding protein that regulates iron homeostasis; refs. 90, 117-120). A common mutation C282Y in HFE-β_2M results in loss of β_2-M association and iron loading in hemochromatosis patients (120). In contrast to all other MHC-like proteins, ZAG and MIC-A (a divergent member of the MHC family) are not found in association with β_2-M as a light chain (15, 90). The close resemblance of ZAG at the structure level, one would reasonably expect a role for ZAG in immunity. The most plausible scenario for the involvement of ZAG in the immune system would be through its capacity to bind and present a lipidic entity to T cells, which is not dissimilar from the interaction involving another nonconventional MHC class I molecule, CD1, with natural killer T cells (121). Recently, Rolli et al. (12) determined that immune system does not seem to be significantly altered in the absence of ZAG, although they cannot deny the role of ZAG in the immunoregulation.

Cell Adhesion

Due to the presence of Arg-Gly-Asp (residues 231-233) in α3 domain, it may attain the properties of cell adhesion between cells and extracellular matrices (102). Based on the RGDV sequence, Takagaki et al. (122) showed the attachment and spreading of various cells on ZAG-coated plates, and they found very less cell spreading compared with fibronectin, laminin, fibrinogen, and vitronectin. Interestingly, the cell spreading for serum and seminal ZAG was the same despite many posttranslational differences. Furthermore, they used RGD-containing synthetic peptides and observed appreciable inhibition in cell spreading and suggested that RGD region of ZAG plays an important role in cell spreading (122). Integrin-mediated cell adhesion requires divalent (Mg^{2+}) cations (123), and interestingly, ZAG also shows adhesion only in the presence of Mg^{2+} and Mn^{2+}. This suggests the role of ZAG in the integrin-mediated adhesion. It was further observed that synthetic peptides, such as RGDS and ELRGDV, were recognized by integrin ($\alpha_5\beta_1$ and $\alpha_v\beta_3$), suggesting that the RGD region of the ZAG interacts with integrin $\alpha_v\beta_3$ molecule on the cell surface of MC3T3-E1 cells (124-126). These studies also reported that the MC3T3-E1 (mouse osteoblastic-like) cells adhered to the ZAG-coated surface and showed that the vitronectin receptor takes part in ZAG-mediated cell adhesion. Furthermore, it was concluded that low molecular weight kininogen and its derivatives promote ZAG-mediated MC3T3-E1 cell adhesion and that domain α3 of the common heavy chain of both kininogens is responsible for the acceleration (126). Finally, Ogikubo et al. (126) identified the amino acid sequence accelerating ZAG-mediated MC3T3-E1 cell adhesion and suggested that this region mainly interacted with the cell surface integrins to regulate cell attachment and spreading. Cell attachment to ZAG is comparable with that for fibronectin and is inhibited by the synthetic RGD peptides RGD, RGDV, and RGDS (102).

RNase Activity

ZAG inhibits the proliferation of oral tumor cell line due to its RNase activity. The RNase activity may be important to the physiologic function of ZAG. ZAG is widely distributed in various organs and body fluids. In these organs, the biological activity of ZAG has been associated with infection, immunoregulation, and antitumor activity. Lei et al. (127) compared the

RNase activity of ZAG with two prominent RNases, such as human pancreatic RNase A and frog oocyte onconase. Surprisingly, all the three RNases show several similarities despite structural and catalytic differences. All three RNase are pyrimidine specific, but RNase A shows a strong preference for polycytidylic acid and much less for polyuridylic acid (128) and onconase prefers polyuridylic acid (129). ZAG responds equally to polycytidylic acid and polyuridylic acid. The specific activity of ZAG is similar to that of onconase and is 2 orders of magnitude lower than that of RNase A. Like onconase, ZAG also has pyroglutamine at its NH_2 terminus, which is required for its RNase activity (129). As a soluble molecule, ZAG is likely to degrade and eliminate by-products of RNA processing reactions within cells, as well as incorrectly synthesized or damaged RNA. Degraded RNA may supply cell nutrients for the continual readjustment of the RNA pools to the changing needs of the cell (130).

Lipid Mobilization

ZAG is responsible for the lipid catabolism seen in cancer patients (68). Human LMF is homologous to serum ZAG in amino acid sequence, electrophoretic mobility, and immunoreactivity, which leads to the same biological activity (3). Due to its high level of amino acid sequence identity to tumor-derived LMF, which is associated with the dramatic loss of adipose tissues in cancer cachexia, ZAG has been shown to stimulate lipid loss by adipocytes both *in vivo* and *in vitro* (4, 37). The lipolysis is regulated by reversible phosphorylation at a single serine residue, which is catalyzed by cAMP-dependent protein kinase A (131). The concentration of cAMP in adipocyte directly affects the rate of lipolysis, which is mediated by hormones and other substances. Likely, both LMF and ZAG have been shown to stimulate lipolysis in isolated murine epididymal adipocytes. This stimulation is associated with an activation of adenylate cyclase in adipocyte plasma membranes in a GTP-dependent manner (3, 132). Loss of body weight could be attributed exclusively to the loss of body fat, which seems to be due to the lipolytic activity of ZAG when combined effect of uncoupling protein was studied. Finally, it was suggested that ZAG may be useful for the treatment of obesity (4). Recently, the same group provided an alternative mechanism by which eicosapentaenoic acid may preserve adipose mass in cachexia. Glucocorticoid-mediated up-regulation of ZAG expression in white and brown adipose tissues leads to lipolysis (37). The mechanism by which eicosapentaenoic acid interferes with the up-regulation of ZAG expression by glucocorticoids has not been evaluated, but a potential mechanism is through Sp1-binding sites on DNA (133). It was also suggested that the permissive effect of glucocorticoids toward lipolysis may result from an increase in ZAG expression (134). Induction of lipolysis by dexamethasone is attenuated by anti-ZAG antisera, and treatment of adipocytes with dexamethasone increases ZAG expression in both cells and medium, suggesting that the up-regulation of ZAG in cachexia may be due to elevated circulatory glucocorticoids (46). In a recent study, the ZAG knockout mice were used to see the role of ZAG in body fat depletion and it was found that mice were overweight with respect to wild-type littermates inferring the opposite [i.e., ZAG overexpression should lead to weight loss and, eventually, cachexia (12)]. The possible role of ZAG has been proposed in the regulation of body weight and age-dependent changes in genetically influenced obesity (135).

Regulation of Melanin Production

The role of ZAG in melanin production was determined using B16 tumor cell line. This cell line, which was either transfected to express ZAG strongly or treated with exogenous ZAG, has shown a decreased melanin production *in vitro* due to decreased levels of tyrosinase protein and minimal tyrosinase activity, which is the key step for melanin production (136). ZAG inhibits melanin synthesis in B16 cells at a posttranscriptional level. Furthermore, ZAG seems to decrease tumor cell growth and melanin synthesis *in vivo* more strongly relative to its effects *in vitro*, suggesting that ZAG has a different mechanism *in vivo* and *in vitro*. Despite its inhibitory activity, the minimal level of ZAG is also required for melanin synthesis. As previously reported, tumor necrosis factor-α also inhibits melanin synthesis by human primary melanoma and B16 melanoma cells (137, 138), but ZAG inhibits melanin synthesis by B16 cells via a tumor necrosis factor–independent mechanism. ZAG is produced by normal epidermal keratinocytes, where its expression increases with cellular differentiation (29). It is also shown that keratinocyte-derived factors influence melanocyte behavior, including melanocyte proliferation, dendricity, and total melanin production (139, 140).

Hindering Tumor Proliferation

ZAG also down-regulates cyclin-dependent kinase, which is responsible for regulating G_2-M transition, a rate-limiting step in the cell cycle, suggesting that ZAG indirectly plays a role in hindering tumor progression (103). ZAG belongs to the macroglobulin family, an ancient and evolutionarily conservative link of the immune system, which is actively involved in both inhibition of tumor growth and proliferation. The capacity of macroglobulins for binding hydrolases makes it possible to inhibit the enzyme-mediated tumor invasion (141). At the same time, an excess of macroglobulin/hydrolase complexes can activate apoptosis (142). The tumor is able of using macroglobulins, especially pregnancy-associated proteins, for its own protection (142, 143). Specifically, pregnancy-associated α2-glycoprotein, which is actively produced by human tumor cells, blocks the MHCs. Nevertheless, the actively growing tumor expresses many receptors to macroglobulins, which are the main carriers of some cytokines and growth factors essential for proliferation.

Similarities with Classic MHC-I Molecules

Human ZAG shows a remarkable sequence identities with MHC-I molecules and especially the residues that are responsible for the antigen binding and are highly conserved (13). ZAG shares about 30% to 47% sequence homology with classic MHC-I–like molecules, which play a major role in antigen presentation and processing by cytotoxic T cells (144, 145). Like MHC-I, ZAG consists of three sets of structural homologous domains (α1, α2, and α3), except the difference

occurring at proximal membrane anchored part due to extra 13 COOH-terminal residues that are absent in ZAG (11). Multiple sequence alignment of ZAG with classic antigen-binding molecules, such as MHC-I, HLAs, FcRn, HFE, and CD1, shows that most of the antigen-binding residues are conserved (Table 2). Interestingly, all the proteins have four conserved cysteine residues, which form disulfide linkages. The residues involved in the binding to β_2M and antigenic peptide are also conserved. The structure of ZAG (PDB: 2ICN) superimposed with heavy chain of HLA (PDB: 2BCK), CD1 (PDB: 1CD1), HFE (PDB 1A6Z), FcRn (PDB: 3FRU), and GDT (PDB: 1C16) is quite similar in structure organization and fold to each other despite very low sequence identity (20-37%). The antigen-binding cleft of ZAG is narrower and flattened. In case of MHC-I, however, it is deeper and broader (Fig. 3A). The groove is deepest in CD1 (Fig. 3B), which specifically binds to hydrophobic antigens such as lipids, lipoproteins, and hydrophobic peptides. The groove, however, is narrowest in case of FcRn (Fig. 3C) and broadest in HFE (Fig. 3D). Although their structure and fold α3 domain are quite similar, ZAG does not associate with TCR and CD8. It is, however, not possible to rule out an interaction between ZAG and TCR because the class I MHC residues that make contact with this receptor are not particularly conserved. Single substitution of the α3 domain at position Trp^{245} is solely responsible for CD8 binding (146). The COOH-terminal region of α3 domain of MHC-I is directly involved in the interaction with CD8. Comparisons among the other point mutations within the α3 domain of MHC-I have identified further positions within the loop between strands 3 and 4 and the strand 4 that affect CD8 binding. The loop residues (220-229) of MHC-I contain five acidic residues, which are important for a CD8 binding,

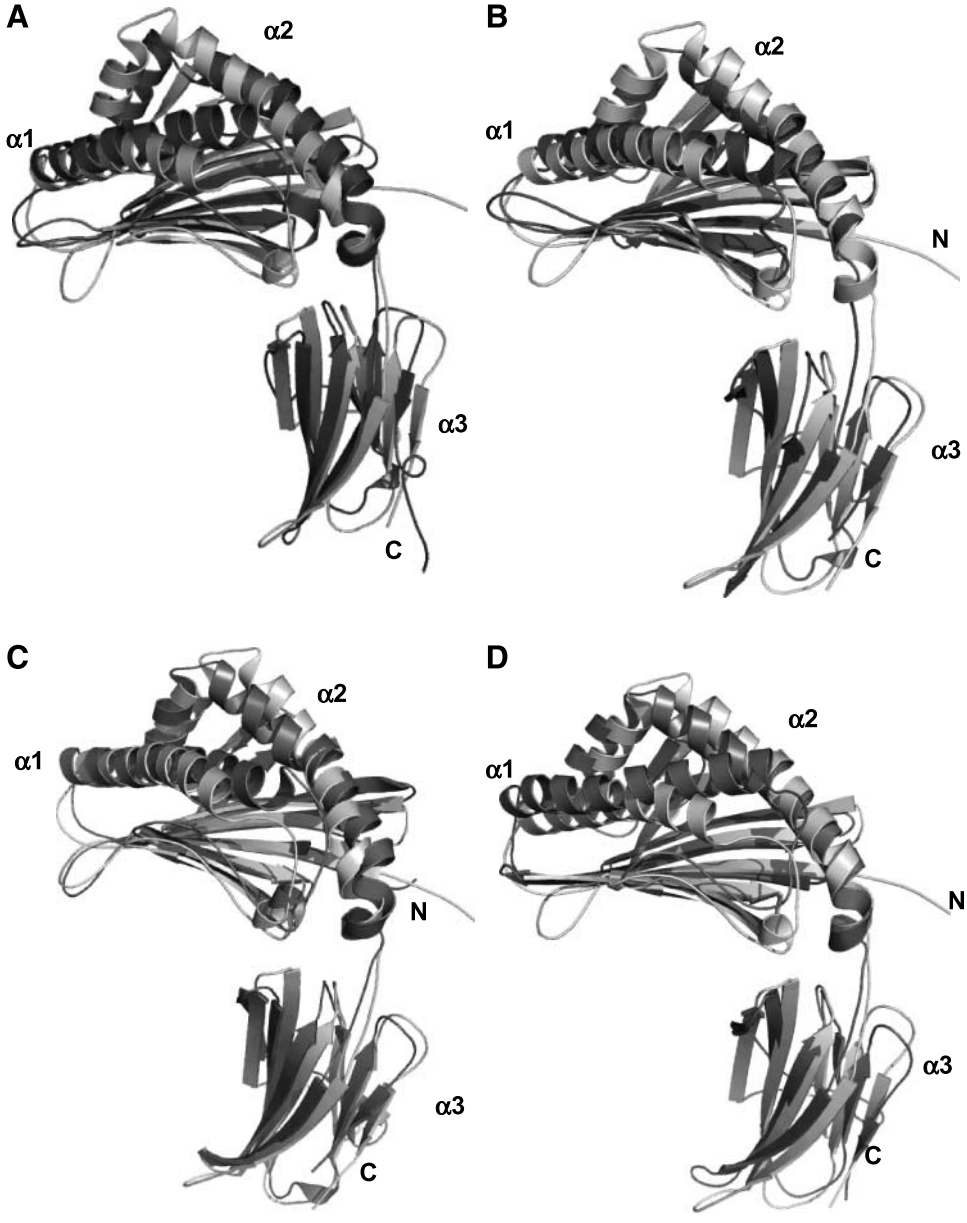


FIGURE 3. Superposition of ZAG (*light gray*) with classic MHC-I molecules (*dark gray*): HLA-A (**A**), HFE (**B**), FcRn (**C**), and CD1 (**D**).

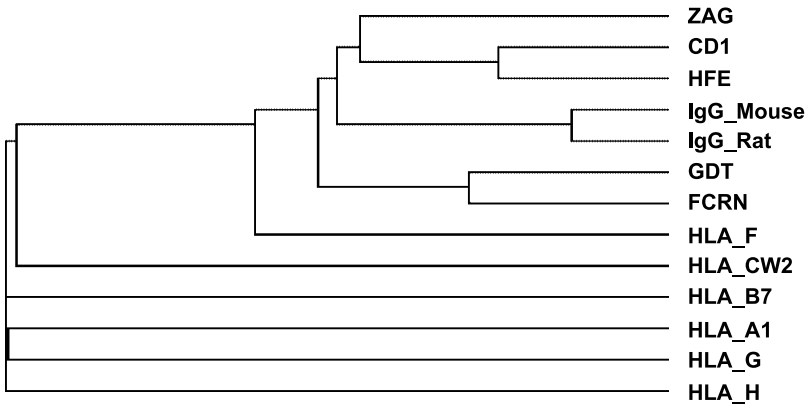


FIGURE 4. Phylogenetic tree of ZAG.

suggesting that negatively charged loop may be a central site for the CD8 contact (147). In the case of ZAG, the loop consists of predominantly hydrophobic residues and only three acidic residues. Due to these mutations in ZAG, it has lost its binding capacity to TCR and consequently failed for antigen processing and presentation.

Phylogenetic Analysis

The origin and evolution of ZAG was analyzed through generating a phylogenic tree using the sequence of ZAG, classic MHC-I molecules, and immunoglobulin heavy chain (Fig. 4). The amino acid sequences were aligned using the ClustalW program (GCG). The resultant alignment was used in the construction of the phylogenetic tree by the neighbor-joining method (MEGA v1.03; Pennsylvania State University, 1993; ref. 148). It is evident from the sequence and phylogenetic analysis that gene duplication in ZAG and classic MHC-I proteins is common (149). The *ZAG* gene was originated by a putative duplication in the gene of class I MHC ancestor occurring at least ~60 million years ago, which was followed by insertion of Alu elements (31). During the course of evolution, ZAG has adapted itself to bind some nonpolymorphic substances and transport them to plasma. MHC-I, however, adapted for the antigen presentation. Furthermore, both ZAG and MHC-I originated from immunoglobulin protein superfamily separately, which has characteristics of transport and recognition (150). The present genomic and phylogenetic analysis suggests that human ZAG, CD1, and HLA class I genes and duplicons originated from the same immunoglobulin-like protein ancestor for different functions.

Conclusions

This is the first time that common putative features of ZAG have been reviewed. Due to potentially exciting and proposed functions, ZAG may be considered as a multidisciplinary functional protein. There are many recent literatures dealing with genetic, biochemical, and functional aspects for this protein. Many questions, however, are still unanswered about its functional role(s) in human serum and semen, particularly with fertilization and immune response. Various proposed roles of ZAG in human body are supported by its structural-functional relationship available in literatures. ZAG is used as biomarker for identification of female breast and male prostatic tumors. The transcriptional regulation of ZAG by androgen reveals its direct role in tumor progression as observed for other tumor-proliferating proteins such as prostate-specific antigen, prostatic acid phosphatase, apolipoprotein D, and progastriscin. The potential multiple functions of ZAG are not only a sensitive biomarker of tumor proliferation but it is also attributed to several functions such as regulation of melanin production, RNase activity, and transport. Moreover, ZAG may be a potential therapeutic target, with its overexpression and under-expression being either beneficial or deleterious.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Burgi W, Schmid K. Preparation and properties of Zn-α2-glycoprotein of normal human plasma. J Biol Chem 1961;236:1066–74.

2. Tada T, Ohkubo I, Niwa M, et al. Immunohistochemical localization of Zn-α2-glycoprotein in normal human tissues. J Histochem Cytochem 1991;39:1221–6.

3. Hirai K, Hussey HJ, Barber MD, et al. Biological evaluation of a lipid-mobilizing factor isolated from the urine of cancer patients. Cancer Res 1998;58:2359–65.

4. Russell ST, Zimmerman TP, Domin BA, Tisdale MJ. Induction of lipolysis *in vitro* and loss of body fat *in vivo* by zinc-α2-glycoprotein. Biochim Biophys Acta 2004;1636:59–68.

5. Bao Y, Bing C, Hunter L, et al. Zinc-α2-glycoprotein, a lipid mobilizing factor, is expressed and secreted by human (SGBS) adipocytes. FEBS Lett 2005;579:41–7.

6. Bing C, Bao Y, Jenkins J, et al. Zinc-α2-glycoprotein, a lipid mobilizing factor, is expressed in adipocytes and is up-regulated in mice with cancer cachexia. Proc Natl Acad Sci U S A 2004;101:2500–5.

7. Diez-Itza I, Sanchez LM, Allende MT, et al. Zn-α2-glycoprotein levels in breast cancer cytosols and correlation with clinical, histological and biochemical parameters. Eur J Cancer 1993;29A:1256–60.

8. Freije JP, Fueyo A, Uria J, Lopez-Otin C. Human Zn-α2-glycoprotein cDNA cloning and expression analysis in benign and malignant breast tissues. FEBS Lett 1991;290:247–9.

9. Frenette G, Dube JY, Lazure C, et al. The major 40-kDa glycoprotein in human prostatic fluid is identical to Zn-α2-glycoprotein. Prostate 1987;11:257–70.

10. Araki T, Gejyo F, Takagaki K, et al. Complete amino acid sequence of human plasma Zn-α2-glycoprotein and its homology to histocompatibility antigens. Proc Natl Acad Sci U S A 1988;85:679–83.

11. Sanchez LM, Chirino AJ, Bjorkman P. Crystal structure of human ZAG, a fat-depleting factor related to MHC molecules. Science 1999;283:1914–9.

12. Rolli V, Radosavljevic M, Astier V, et al. Lipolysis is altered in MHC class I zinc-α(2)-glycoprotein deficient mice. FEBS Lett 2007;581:394–400.

13. McDermott LC, Freel JA, West AP, et al. Zn-α2-glycoprotein, an MHC class I-related glycoprotein regulator of adipose tissues: modification or abrogation of ligand binding by site-directed mutagenesis. Biochemistry 2006; 45:2035–41.

14. Kennedy MW, Heikema AP, Cooper A, et al. Hydrophobic ligand binding by Zn-α2-glycoprotein, a soluble fat-depleting factor related to major histocompatibility complex proteins. J Biol Chem 2001;276:35008–13.

15. Sanchez LM, Lopez-Otin C, Bjorkman PJ. Biochemical characterization and crystalization of human Zn-α2-glycoprotein, a soluble class I major histocompatibility complex homolog. Proc Natl Acad Sci U S A 1997;94:4626–30.

16. Schwick HG, Haupt H. Purified human plasma proteins of unknown function. Jpn J Med Sci Biol 1981;34:299–327.

17. Ohkubo I, Niwa M, Takashima A, et al. Human seminal plasma Zn-α2-glycoprotein: its purification and properties as compared with human plasma Zn-α2-glycoprotein. Biochim Biophys Acta 1990;1034:152–6.

18. Poortmans JR, Schmid K. The level of Zn-α2-glycoprotein in normal human body fluids and kidney extract. J Lab Clin Med 1968;71:807–11.

19. Jirka M, Blanicky P. Polymorphism of human serum Zn-α2-glycoprotein and its behaviour during ontogenesis using quantitative crossed starch gel immunoelectrophoresis. Clin Chim Acta 1980;103:61–6.

20. Ghafouri B, Tagesson C, Lindahl M. Mapping of proteins in human saliva using two-dimensional gel electrophoresis and peptide mass fingerprinting. Proteomics 2003;3:1003–15.

21. Mazoujian G. Immunohistochemistry of GCDFP-24 and zinc α2 glycoprotein in benign sweat gland tumors. Am J Dermatopathol 1990;12:452–7.

22. Brysk MM, Lei G, Rajaraman S, et al. Gene expression of zinc-α2-glycoprotein in normal human epidermal and buccal epithelia. In Vivo 1997;11: 271–4.

23. Burgi W, Simonen S, Baudner S, Schmid K. Unusually high concentrations of Zn α2-glycoprotein and the lack of α2HS-glycoprotein in human ejaculates. Clin Chem 1989;35:1649–50.

24. Bundred NJ, Miller WR, Walker RA. An immunohistochemical study of the tissue distribution of the breast cyst fluid protein, zinc α2 glycoprotein. Histopathology 1987;11:603–10.

25. Jain S, Rajput A, Kumar Y, et al. Proteomic analysis of urinary protein markers for accurate prediction of diabetic kidney disorder. J Assoc Physicians India 2005;53:513–20.

26. Ding M, Umetsu K, Nakayashiki N, et al. Distribution of human Zn-α-2-glycoprotein types in Chinese and Korean populations. Hum Hered 1990;40: 311–2.

27. Henshall SM, Horvath LG, Quinn DI, et al. Zinc-α2-glycoprotein expression as a predictor of metastatic prostate cancer following radical prostatectomy. J Natl Cancer Inst 2006;98:1420–4.

28. Sanchez LM, Vizoso F, Diez-Itza I, Lopez-Otin C. Identification of the major protein components in breast secretions from women with benign and malignant breast diseases. Cancer Res 1992;52:95–100.

29. Lei G, Arany I, Selvanayagam P, et al. Detection and cloning of epidermal zinc-α2-glycoprotein cDNA and expression in normal human skin and in tumors. J Cell Biochem 1997;67:216–22.

30. Ueyama H, Niwa M, Tada T, et al. Cloning and nucleotide sequence of a human Zn-α2-glycoprotein cDNA and chromosomal assignment of its gene. Biochem Biophys Res Commun 1991;177:696–703.

31. Ueyama H, Deng HX, Ohkubo I. Molecular cloning and chromosomal assignment of the gene for human Zn-α2-glycoprotein. Biochemistry 1993;32: 12968–76.

32. Freije JP, Fueyo A, Uria JA, et al. Human Zn-α2-glycoprotein: complete genomic sequence, identification of a related pseudogene and relationship to class I major histocompatibility complex genes. Genomics 1993;18:575–87.

33. Kaplan DJ, Jurka J, Solus JF, Duncan CH. Medium reiteration frequency repetitive sequences in the human genome. Nucleic Acids Res 1991;19:4731–8.

34. Jurka J, Walichiewicz J, Milosavljevic A. Prototypic sequences for human repetitive DNA. J Mol Evol 1992;35:286–91.

35. Lopez-Boado YS, Diez-Itza I, Tolivia J, Lopez-Otin C. Glucocorticoids and androgens up-regulate the Zn-α2-glycoprotein messenger RNA in human breast cancer cells. Breast Cancer Res Treat 1994;29:247–58.

36. Chalbos D, Haagensen D, Parish T, Rochefort H. Identification and androgen regulation of two proteins released by T47D human breast cancer cells. Cancer Res 1987;47:2787–92.

37. Russell ST, Tisdale MJ. Effect of eicosapentaenoic acid (EPA) on expression of a lipid mobilizing factor in adipose tissue in cancer cachexia. Prostaglandins Leukot Essent Fatty Acids 2005;72:409–14.

38. Russell ST, Tisdale MJ. The role of glucocorticoids in the induction of zinc-α2-glycoprotein expression in adipose tissue in cancer cachexia. Br J Cancer 2005;92:876–81.

39. Gangadharan B, Antrobus R, Dwek RA, Zitzmann N. Novel serum biomarker candidates for liver fibrosis in hepatitis C patients. Clin Chem 2007; 53:1792–9.

40. Varghese SA, Powell TB, Budisavljevic MN, et al. Urine biomarkers predict the cause of glomerular disease. J Am Soc Nephrol 2007;18:913–22.

41. Irmak S, Tilki D, Heukeshoven J, et al. Stage-dependent increase of orosomucoid and zinc-α2-glycoprotein in urinary bladder cancer. Proteomics 2005;5:4296–304.

42. Lauritzen B, Lykkesfeldt J, Skaanild MT, et al. Putative biomarkers for evaluating antibiotic treatment: an experimental model of porcine *Actinobacillus pleuropneumoniae* infection. Res Vet Sci 2003;74:261–70.

43. Abdul-Rahman PS, Lim B, Hashim OH. Expression of high-abundance proteins in sera of patients with endometrial and cervical cancers: analysis using 2-DE with silver staining and lectin detection methods. Electrophoresis 2007;28: 1989–96.

44. Hale LP, Price DT, Sanchez LM, et al. Zinc α-2-glycoprotein is expressed by malignant prostatic epithelium and may serve as a potential serum marker for prostate cancer. Clin Cancer Res 2001;7:846–53.

45. Kamoshida S, Watanabe K, Suzuki M, et al. Expression of cancer cachexia-related factors in human cancer xenografts: an immunohistochemical analysis. Biomed Res 2006;27:275–81.

46. Knapp ML, al-Sheibani S, Riches PG, et al. Hormonal factors associated with weight loss in patients with advanced breast cancer. Ann Clin Biochem 1991;28: 480–6.

47. Gagnon S, Tetu B, Dube JY, Tremblay RR. Expression of Zn-α2-glycoprotein and PSP-94 in prostatic adenocarcinoma. An immunohistochemical study of 88 cases. Am J Pathol 1990;136:1147–52.

48. Lapointe J, Li C, Higgins JP, et al. Gene expression profiling identifies clinically relevant subtypes of prostate cancer. Proc Natl Acad Sci U S A 2004; 101:811–6.

49. Hull GW, Rabbani F, Abbas F, et al. Cancer control with radical prostatectomy alone in 1,000 consecutive patients. J Urol 2002;167:528–34.

50. Descazeaud A, de la Taille A, Allory Y, et al. Characterization of ZAG protein expression in prostate cancer using a semi-automated microscope system. Prostate 2006;66:1037–43.

51. Hassan MI, Kumar V, Kashav T, et al. Proteomic approach for the purification of major seminal plasma proteins involved in tumor proliferation. J Sep Sci 2007;30:1979–88.

52. Vizoso F, Sanchez LM, Diez-Itza I, et al. Factors affecting protein composition of breast secretions from nonlactating women. Breast Cancer Res Treat 1992;23:251–8.

53. Ring AE, Zabaglo L, Ormerod MG, et al. Detection of circulating epithelial cells in the blood of patients with breast cancer: comparison of three techniques. Br J Cancer 2005;92:906–12.

54. Mrusek S, Classen-Linke I, Vloet A, et al. Estradiol and medroxyprogesterone acetate regulated genes in T47D breast cancer cells. Mol Cell Endocrinol 2005;235:39–50.

55. Bundred NJ, Scott WN, Davies SJ, et al. Zinc α-2 glycoprotein levels in serum and breast fluids: a potential marker of apocrine activity. Eur J Cancer 1991;27:549–52.

56. Bundred NJ, Walker RA, Everington D, et al. Is apocrine differentiation in breast carcinoma of prognostic significance? Br J Cancer 1990;62:113–7.

57. Lopez-Otin C, Diamandis EP. Breast and prostate cancer: an analysis of common epidemiological, genetic, and biochemical features. Endocr Rev 1998; 19:365–96.

58. Sanders PM, Tisdale MJ. Effect of zinc-α2-glycoprotein (ZAG) on expression of uncoupling proteins in skeletal muscle and adipose tissue. Cancer Lett 2004;212:71–81.

59. Brysk MM, Lei G, Adler-Storthz K, et al. Zinc-α2-glycoprotein expression as a marker of differentiation in human oral tumors. Cancer Lett 1999;137: 117–20.

60. Mazoujian G. Apocrine carcinoma of the breast. Am J Clin Pathol 1990;94: 485–6.

61. García-Ramírez M, Canals F, Hernández C, et al. Proteomic analysis of human vitreous fluid by fluorescence-based difference gel electrophoresis (DIGE): a new strategy for identifying potential candidates in the pathogenesis of proliferative diabetic retinopathy. Diabetologia 2007;50:940–6.

62. Ding Z, Qu F, Guo W, et al. Identification of sperm forward motility-related proteins in human seminal plasma. Mol Reprod Dev 2007;74:1124–31.

63. Steuber T, Helo P, Lilja H. Circulating biomarkers for prostate cancer. World J Urol 2007;25:111–9.

64. Cordon-Cardo C, Kotsianti A, Verbel DA, et al. Improved prediction of prostate cancer recurrence through systems pathology. J Clin Invest 2007;117:1876–83.

65. Veveris-Lowe TL, Kruger SJ, Walsh T, et al. Seminal fluid characterization for male fertility and prostate cancer: kallikrein-related serine proteases and whole proteome approaches. Semin Thromb Hemost 2007;33:87–99.

66. Morley JE, Thomas DR, Wilson MG. Cachexia: pathophysiology and clinical relevance. Am J Clin Nutr 2006;83:735–43.

67. Morley JE, Baumgartner R. Cytokine-related aging process. J Gerontol A Biol Sci Med Sci 2004;59:M924–9.

68. Todorov PT, McDevitt TM, Meyer DJ, et al. Purification and characterization of a tumor lipid-mobilizing factor. Cancer Res 1998;58:2353–8.

69. Russell ST, Hirai K, Tisdale MJ. Role of β3-adrenergic receptors in the action of a tumour lipid mobilizing factor. Br J Cancer 2002;86:424–8.

70. Ueyama H, Naitoh H, Ohkubo I. Structure and expression of rat and mouse mRNAs for Zn-α2-glycoprotein. J Biochem (Tokyo) 1994;116:677–81.

71. Fueyo A, Uria JA, Freije JM, Lopez-Otin C. Cloning and expression analysis of the cDNA encoding rat Zn-α2-glycoprotein. Gene 1994;145:245–9.

72. Delker SL, West AP, Jr., McDermott L, et al. Crystallographic studies of ligand binding by Zn-α2-glycoprotein. J Struct Biol 2004;148:205–13.

73. Hill DM, Kasliwal T, Schwarz E, et al. A dominant negative mutant β2-microglobulin blocks the extracellular folding of a major histocompatibility complex class I heavy chain. J Biol Chem 2003;278:5630–8.

74. Rock KL, Rothstein LE, Gamble SR, Benacerraf B. Reassociation with β2-microglobulin is necessary for Kb class I major histocompatibility complex binding of exogenous peptides. Proc Natl Acad Sci U S A 1990;87:7517–21.

75. Rock KL, Gramm C, Benacerraf B. Low temperature and peptides favor the formation of class I heterodimers on RMA-S cells at the cell surface. Proc Natl Acad Sci U S A 1991;88:4200–4.

76. Kozlowski S, Takeshita T, Boehncke WH, et al. Excess β2 microglobulin promoting functional peptide association with purified soluble class I MHC molecules. Nature 1991;349:74–7.

77. Karpenko V, Kaupova M, Kodicek M. The conformation and stability of human Zn-α2-glycoprotein in aqueous and methanolic solutions. Biophys Chem 1997;69:209–17.

78. Fahnestock ML, Tamir I, Narhi L, Bjorkman PJ. Thermal stability comparison of purified empty and peptide-filled forms of a class I MHC molecule. Science 1992;258:1658–62.

79. Ljunggren HG, Sturmhofel K, Wolpert E, et al. Transfection of β2-microglobulin restores IFN-mediated protection from natural killer cell lysis in YAC-1 lymphoma variants. J Immunol 1990;145:380–6.

80. Hosken NA, Bevan MJ. An endogenous antigenic peptide bypasses the class I antigen presentation defect in RMA-S. J Exp Med 1992;175:719–29.

81. Townsend A, Ohlen C, Foster L, et al. A mutant cell in which association of class I heavy and light chains is induced by viral peptides. Cold Spring Harbor Symp Quant Biol 1989;54 Pt 1:299–308.

82. Ljunggren HG, Stam NJ, Ohlen C, et al. Empty MHC class I molecules come out in the cold. Nature 1990;346:476–80.

83. Orr HT, Lancet D, Robb RJ, et al. The heavy chain of human histocompatibility antigen HLA-B7 contains an immunoglobulin-like region. Nature 1979;282:266–70.

84. Bouvier M, Wiley DC. Importance of peptide amino and carboxyl termini to the stability of MHC class I molecules. Science 1994;265:398–402.

85. Fahnestock ML, Johnson JL, Feldman RM, et al. Effects of peptide length and composition on binding to an empty class I MHC heterodimer. Biochemistry 1994;33:8149–58.

86. Willcox BE, Thomas LM, Bjorkman PJ. Crystal structure of HLA-A2 bound to LIR-1, a host and viral major histocompatibility complex receptor. Nat Immunol 2003;4:913–9.

87. Willcox BE, Thomas LM, Chapman TL, et al. Crystal structure of LIR-2 (ILT4) at 1.8 Å: differences from LIR-1 (ILT2) in regions implicated in the binding of the human cytomegalovirus class I MHC homolog UL18. BMC Struct Biol 2002;2:6.

88. Chapman TL, Heikema AP, West AP, Jr., Bjorkman PJ. Crystal structure and ligand binding properties of the D1D2 region of the inhibitory receptor LIR-1 (ILT2). Immunity 2000;13:727–36.

89. West AP, Jr., Bjorkman PJ. Crystal structure and immunoglobulin G binding properties of the human major histocompatibility complex-related Fc receptor. Biochemistry 2000;39:9698–708.

90. Bennett MJ, Lebron JA, Bjorkman PJ. Crystal structure of the hereditary haemochromatosis protein HFE complexed with transferrin receptor. Nature 2000;403:46–53.

91. Martin WL, Bjorkman PJ. Characterization of the 2:1 complex between the class I MHC-related Fc receptor and its Fc ligand in solution. Biochemistry 1999;38:12639–47.

92. Lebron JA, Bjorkman PJ. The transferrin receptor binding site on HFE, the class I MHC-related protein mutated in hereditary hemochromatosis. J Mol Biol 1999;289:1109–18.

93. Lebron JA, Bennett MJ, Vaughn DE, et al. Crystal structure of the hemochromatosis protein HFE and characterization of its interaction with transferrin receptor. Cell 1998;93:111–23.

94. Burmeister WP, Gastinel LN, Simister NE, et al. Crystal structure at 2.2 Å resolution of the MHC-related neonatal Fc receptor. Nature 1994;372:336–43.

95. Burmeister WP, Huber AH, Bjorkman PJ. Crystal structure of the complex of rat neonatal Fc receptor with Fc. Nature 1994;372:379–83.

96. Gastinel LN, Simister NE, Bjorkman PJ. Expression and crystallization of a soluble and functional form of an Fc receptor related to class I histocompatibility molecules. Proc Natl Acad Sci U S A 1992;89:638–42.

97. Raghavan M, Chen MY, Gastinel LN, Bjorkman PJ. Investigation of the interaction between the class I MHC-related Fc receptor and its immunoglobulin G ligand. Immunity 1994;1:303–15.

98. Madden DR. The three-dimensional structure of peptide-MHC complexes. Annu Rev Immunol 1995;13:587–622.

99. Matsumura M, Fremont DH, Peterson PA, Wilson IA. Emerging principles for the recognition of peptide antigens by MHC class I molecules. Science 1992;257:927–34.

100. Zeng Z, Castano AR, Segelke BW, et al. Crystal structure of mouse CD1: an MHC-like fold with a large hydrophobic binding groove. Science 1997;277:339–45.

101. Collins EJ, Garboczi DN, Wiley DC. Three-dimensional structure of a peptide extending from one end of a class I MHC binding site. Nature 1994;371:626–9.

102. Lei G, Brysk H, Arany I, et al. Characterization of zinc-α(2)-glycoprotein as a cell adhesion molecule that inhibits the proliferation of an oral tumor cell line. J Cell Biochem 1999;75:160–9.

103. He N, Brysk H, Tyring SK, et al. Zinc-α(2)-glycoprotein hinders cell proliferation and reduces cdc2 expression. J Cell Biochem 2001;81:162–9.

104. Geraci F, Giudice G. Factors which influence sperm ability to fertilize. J Submicrosc Cytol Pathol 2005;37:215–22.

105. Jaffe K, Camejo MI, Carrillo TE, et al. Evidence favoring sperm selection over sperm competition in the interaction between human seminal plasma and sperm motility *in vitro*. Arch Androl 2006;52:45–50.

106. Petrunkina AM, Volker G, Brandt H, et al. Functional significance of responsiveness to capacitating conditions in boar spermatozoa. Theriogenology 2005;64:1766–82.

107. Myers K, Somanath PR, Berryman M, Vijayaraghavan S. Identification of chloride intracellular channel proteins in spermatozoa. FEBS Lett 2004;566:136–40.

108. Pal PC, Rajalakshmi M, Manocha M, et al. Semen quality and sperm functional parameters in fertile Indian men. Andrologia 2006;38:20–5.

109. Parker HM, McDaniel CD. The immediate impact of semen diluent and rate of dilution on the sperm quality index, ATP utilization, gas exchange, and ionic balance of broiler breeder sperm. Poult Sci 2006;85:106–16.

110. Jones R. Plasma membrane structure and remodelling during sperm maturation in the epididymis. J Reprod Fertil Suppl 1998;53:73–84.

111. Frenette G, Thabet M, Sullivan R. Polyol pathway in human epididymis and semen. J Androl 2006;27:233–9.

112. Schuh SM, Carlson AE, McKnight GS, et al. Signaling pathways for modulation of mouse sperm motility by adenosine and catecholamine agonists. Biol Reprod 2006;74:492–500.

113. Jha KN, Kameshwari DB, Shivaji S. Role of signaling pathways in regulating the capacitation of mammalian spermatozoa. Cell Mol Biol (Noisy-le-grand) 2003;49:329–40.

114. Shibata S, Miura K. Nephritogenic glycoprotein. IX. Plasma Zn-α2-glycoprotein as a second source of nephritogenic glycoprotein in urine. Nephron 1982;31:170–6.

115. Deutscher MP. Ribonuclease multiplicity, diversity, and complexity. J Biol Chem 1993;268:13011–4.

116. Beckman EM, Porcelli SA, Morita CT, et al. Recognition of a lipid antigen by CD1-restricted $\alpha\beta^{+}$ T cells. Nature 1994;372:691–4.

117. Gross CN, Irrinki A, Feder JN, Enns CA. Co-trafficking of HFE, a nonclassical major histocompatibility complex class I protein, with the transferrin receptor implies a role in intracellular iron regulation. J Biol Chem 1998;273: 22068–74.

118. Parkkila S, Waheed A, Britton RS, et al. Association of the transferrin receptor in human placenta with HFE, the protein defective in hereditary hemochromatosis. Proc Natl Acad Sci U S A 1997;94:13198–202.

119. Waheed A, Parkkila S, Saarnio J, et al. Association of HFE protein with transferrin receptor in crypt enterocytes of human duodenum. Proc Natl Acad Sci U S A 1999;96:1579–84.

120. Waheed A, Parkkila S, Zhou XY, et al. Hereditary hemochromatosis: effects of C282Y and H63D mutations on association with β2-microglobulin, intracellular processing, and cell surface expression of the HFE protein in COS-7 cells. Proc Natl Acad Sci U S A 1997;94:12384–9.

121. Attinger A, Devine L, Wang-Zhu Y, et al. Molecular basis for the high affinity interaction between the thymic leukemia antigen and the CD8αα molecule. J Immunol 2005;174:3501–7.

122. Takagaki M, Honke K, Tsukamoto T, et al. Zn-α2-glycoprotein is a novel adhesive protein. Biochem Biophys Res Commun 1994;201:1339–47.

123. Gailit J, Ruoslahti E. Regulation of the fibronectin receptor affinity by divalent cations. J Biol Chem 1988;263:12927–32.

124. Kuvibidila SR, Sandoval M, Lao J, et al. Plasma zinc levels inversely correlate with vascular cell adhesion molecule-1 concentration in children with sickle cell disease. J Natl Med Assoc 2006;98:1263–72.

125. Uzzo RG, Crispen PL, Golovine K, et al. Diverse effects of zinc on NF-κB and AP-1 transcription factors: implications for prostate cancer progression. Carcinogenesis 2006;27:1980–90.

126. Ogikubo O, Maeda T, Yamane T, et al. Regulation of Zn-α2-glycoprotein-mediated cell adhesion by kininogens and their derivatives. Biochem Biophys Res Commun 1998;252:257–62.

127. Lei G, Arany I, Tyring SK, et al. Zinc-α2-glycoprotein has ribonuclease activity. Arch Biochem Biophys 1998;355:160–4.

128. Sorrentino S, Libonati M. Human pancreatic-type and nonpancreatic-type ribonucleases: a direct side-by-side comparison of their catalytic properties. Arch Biochem Biophys 1994;312:340–8.

129. Boix E, Wu Y, Vasandani VM, et al. Role of the N terminus in RNase A homologues: differences in catalytic activity, ribonuclease inhibitor interaction and cytotoxicity. J Mol Biol 1996;257:992–1007.

130. Koonin EV, Deutscher MP. RNase T shares conserved sequence motifs with DNA proofreading exonucleases. Nucleic Acids Res 1993;21:2521–2.

131. Stralfors P, Olsson H, Belfrange P. Hormone sensitive lipase. In: Boy PD and Krebs EG, editors. The enzymes. New York: Academic Press; 1987. p. 147–77.

132. Tzanavari T, Bing C, Trayhurn P. Postnatal expression of zinc-α(2)-glycoprotein in rat white and brown adipose tissue. Mol Cell Endocrinol 2007; 279:26–33.

133. Clarke SD. Polyunsaturated fatty acid regulation of gene transcription: a molecular mechanism to improve the metabolic syndrome. J Nutr 2001;131: 1129–32.

134. Lacasa D, Agli B, Giudicelli Y. Permissive action of glucocorticoids on catecholamine-induced lipolysis: direct "*in vitro*" effects on the fat cell β-adrenoreceptor-coupled-adenylate cyclase system. Biochem Biophys Res Commun 1988;153:489–97.

135. Gohda T, Makita Y, Shike T, et al. Identification of epistatic interaction involved in obesity using the KK/Ta mouse as a type 2 diabetes model: is Zn-α2 glycoprotein-1 a candidate gene for obesity? Diabetes 2003;52:2175–81.

136. Hale LP. Zinc α-2-glycoprotein regulates melanin production by normal and malignant melanocytes. J Invest Dermatol 2002;119:464–70.

137. Swope VB, Abdel-Malek Z, Kassem LM, Nordlund JJ. Interleukins 1α and 6 and tumor necrosis factor-α are paracrine inhibitors of human melanocyte proliferation and melanogenesis. J Invest Dermatol 1991;96:180–5.

138. Martinez-Esparza M, Jimenez-Cervantes C, Solano F, et al. Mechanisms of melanogenesis inhibition by tumor necrosis factor-α in B16/F10 mouse melanoma cells. Eur J Biochem 1998;255:139–46.

139. Gordon PR, Gilchrest BA. Human melanogenesis is stimulated by diacylglycerol. J Invest Dermatol 1989;93:700–2.

140. Gordon PR, Mansur CP, Gilchrest BA. Regulation of human melanocyte growth, dendricity, and melanization by keratinocyte derived factors. J Invest Dermatol 1989;92:565–72.

141. Zorin NA, Zorina VN, Zorina RM. [Role of proteins of the macroglobulin family in regulation of tumor growth]. Ontogenez 2006;37:12–9.

142. Zorin NA, Zorina VN, Zorina RM. [The role of macroglobulin family proteins in the regulation of inflammation]. Biomed Khim 2006;52:229–38.

143. Zorina VN, Predeina EM, Zorina RM, et al. [The level of pregnancy-associated α2-glycoprotein and the hormonal background during different options of hormonal replacement therapy in the menopausal syndrome]. Klin Lab Diagn 2007;7:24–7.

144. O'Callaghan CA, Tormo J, Willcox BE, et al. Structural features impose tight peptide binding specificity in the nonclassical MHC molecule HLA-E. Mol Cell 1998;1:531–41.

145. Lehner PJ, Cresswell P. Processing and delivery of peptides presented by MHC class I molecules. Curr Opin Immunol 1996;8:59–67.

146. Rudolph MG, Stanfield RL, Wilson IA. How TCRs bind MHCs, peptides, and coreceptors. Annu Rev Immunol 2006;24:419–66.

147. Ibegbu CC, Kennedy MS, Maddon PJ, et al. Structural features of CD4 required for binding to HIV. J Immunol 1989;142:2250–6.

148. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 1987;4:406–25.

149. Kulski JK, Dunn DS, Gaudieri S, et al. Genomic and phylogenetic analysis of the human CD1 and HLA class I multicopy genes. J Mol Evol 2001;53:642–50.

150. Williams AF, Barclay AN. The immunoglobulin superfamily—domains for cell surface recognition. Annu Rev Immunol 1988;6:381–405.