



Understanding Clinical Significance of GPI Anchored Proteins in Mammalian System: Special Emphasis on Genetic Disorders of GPI Anchor Biosynthesis Pathway

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ABSTRACT

The mode of attaching the membrane protein with GPI anchor is known as GPI anchoring of protein and proteins thus formed are known as GPI anchored protein. GPI anchor is a glycolipid moiety which is synthesized in endoplasmic reticulum of cell with the help of a multistep pathway called GPI anchor biosynthesis pathway. This pathway is regulated by more than 26 enzymes and mutation in genes of this pathway leads to disorders called Inherited Genetic Disorders (IGD) which are inherited as recessive trait. This study describes the clinical significance of GPI anchored proteins in mammalian system. **Methods:** This review is focused on different types of inherited genetic disorders caused by mutations in different genes of GPI anchor biosynthesis pathway and also explains the role of GPI anchored proteins in deadly disease like cancer and neurodegenerative prion disease. **Results:** Genetic mutations in GPI anchor biosynthesis pathway lead to disorders called Inherited Genetic Disorders (IGD) which are inherited as recessive trait. The major symptoms of these disorders are intellectual disability, coarse facial features and organs abnormality. Different subunits of GPI transamidase complex, one of the enzyme of this pathway are also identified as oncogenes in different types of cancers. **Conclusion:** Although this pathway is not essential in mammals but genetic defects result in synthesis of defective GPI anchored proteins which lead to many disorders and syndromes.

Keywords: Inherited genetic disorder (IGD), Paroxysmal nocturnal haemoglobinuria, Glypican, Urokinase plasminogen activated receptor, Prion

Abbreviations: GPI: Glycosylphosphatidylinositol; PNH: Paroxysmal Nocturnal Haemoglobinuria; HSC: Hematopoietic Stem Cells; IGD: Inherited Glycosylation Disorder; GPI-T: GPI Transamidase Complex; PIG: Phosphatidylinositol Glycan; PGAP: Post GPI Attachment to Protein; ECM; Extracellular Matrix

INTRODUCTION

Out of different membrane proteins which span the lipid bilayer some proteins are anchored at their C-terminal to a glycolipid moiety called GPI anchor and are tethered to the lipid bilayer [1]. Such proteins are designated as GPI anchored protein and are synthesized through post translational modification that takes place in endoplasmic reticulum. It consists of three different moieties phosphatidylinositol, glycan core and phosphoethanolamine through which the protein is covalently attached. GPI-AP are raft associated proteins and can be released from the membrane with the help of GPI cleaving enzymes [2,3].

More than 150 proteins are GPI anchored in human beings and play a special role at cell surface in the form of blood group antigens, complement regulatory protein CD55, CD59, enzymes, receptors, adhesions. GPI anchor biosynthesis pathway is governed by at least 27 enzymes which are involved in synthesis and transport of GPI anchored proteins at cell surface. Those involved in biosynthesis are designated as PIG genes while those involved in transport are designated as PGAP [3].

Clinical implications of GPI anchored proteins

Although GPI-AP biosynthesis is not indispensable to mammals but any genetic defect leads to glycosylation disorders which are inherited and are known as Inherited Glycosylation Disorders (IGD) [3,4]. Earlier it was thought that

Paroxysmal nocturnal haemoglobinuria is the only disorder caused by somatic mutation in GPI anchor biosynthesis pathway but with the discovery of next generation sequencing identification of genetic causes and the clinical phenotypes of disorders caused by mutations in different genes of GPI anchor biosynthesis pathway has become possible [4]. Till now there are 13 such mutations known in different genes of GPI anchor biosynthesis pathway which are known to cause IGDs [5]. The following section gives a brief description of all Inherited Genetic Disorders in human beings.

By exome sequencing it was found that homozygous and compound heterozygous mutations in PIG-L, results in CHIMES syndrome also known as Zurich neuroectodermal syndrome. It is a recessive autosomal multisystemic disorder characterized by colobomas, ear, heart problems, ichthyosiform dermatosis and intellectual disabilities. The mutated cell lines derived from these patients contain reduced level of GPI anchor markers like CD55 [6]. PIG-L and PIG-Y, two subunits of GPI anchor biosynthesis pathway, are found to be transcriptionally silenced in Burkitt lymphoma cell line [7].

Biallelic mutation in PIG-W causes HPMRS/Mabry syndrome characterized by constant elevated levels of alkaline phosphatase, developmental delay, seizures, coarse facial features and multiple anomalies [8]. Surface expression of two GPI anchored proteins CD16 and CD24 on blood granulocytes is also decreased [3,9].

Single base substitution in promoter region of PIG-M causes homozygous mutation resulting in disruption of Sp1 binding motif and partial decrease in expression of GPI anchored proteins on the surface of granulocytes, B-lymphoblastoid cells and skin fibroblasts. This Inherited Glycosylation Disorder (IGD) is characterized by seizures and thrombosis of hepatic veins. The surface expression of GPI-AP is restored by treatment with sodium butyrate, which inhibited histone deacetylase and increased the acetylation and transcription of PIG-M [3,4,10,11].

Exome sequencing of DNA samples identified a mutation in PIG-V causing Hyperphosphatasia with mental retardation syndrome1 (HPMRS1) also known as Mabry syndrome. This syndrome is an autosomal recessive disorder characterized by mental retardation and elevated serum alkaline phosphatase level. Other clinical symptoms of this disorder are seizures, facial dysmorphism, etc. This mutation also results in reduced surface expression of GPI anchored protein CD16 on blood granulocytes [3,12].

Homozygous mutation in PIG-N causes autosomal recessive syndrome, which is characterized by dysmorphic features, neurological impairments, and cornea [3,13]. Heterozygous mutation in PIG-N also results in syndrome characterized by congenital anomalies, developmental delay, hypotonia and seizures. This deficiency is now known as MCAHS1 (multiple congenital anomalies with hypotonia and seizures [3].

Exome sequencing of DNA samples taken from different individuals suffering from HPMRS resulted in the identification of syndrome caused by mutations in PIG-O. This syndrome is characterized by elevated levels of alkaline phosphatase, facial dysmorphism and seizures leading to early death [14]. HPMRS caused by PIG-O mutation is known as HPMRS2 and results in decreased surface expression of GPI anchored protein CD16 [3,9].

Deficiency in PIG-A also causes inherited disorder termed as MCAHS type 2, where males suffer from neurodegeneration, cutaneous abnormalities and systemic iron overload while children suffer from developmental delay, facial dysmorphism, accelerated linear growth, central nervous system, abnormalities and mildly elevated serum alkaline phosphatase [3]. Similarly, PIG-T deficiency causes MCAHS type 3 disease, where affected individuals show intellectual disability, hypotonia, seizures, skeletal, endocrine, ophthalmologic abnormalities and hypophosphatasia rather than hyperphosphatasia [3,15].

Although deficiency in PGAP1 is compatible with life, but it mainly affects neuronal cells with major clinical symptoms like intellectual disability, developmental delay, encephalopathy, cerebral visual impairment, hypotonia and seizures. The transport of GPI-AP from ER to Golgi cells is slowed down due to attached inositol ring. Moreover, such proteins are resistant to GPI cleaving enzymes and their interaction with putative GPI anchored proteins is also affected [9].

Deficiency in PGAP2 results in HPMRS/Mabry syndrome termed as HPMRS3. Although the affected individuals show intellectual disabilities, hyperphosphatasia, seizures, hypotonia, anorectal abnormality, heart defect, hearing impairment etc but the level of GPI anchored proteins CD55 and CD59 on lymphoblastoid cells are not affected [3,16].

Deficiency in PGAP3 also causes HPMRS/Mabry syndrome termed as HPMRS4. It is a loss of function mutations which results in intellectual disability, developmental delay and hyperphosphatasia. Surface expression of GPI anchored proteins CD16 and CD59 on blood granulocytes are also found to be affected [9].

Paroxysmal nocturnal haemoglobinuria

Paroxysmal nocturnal haemoglobinuria (PNH) is a rare haemolytic disorder characterized by absence of GPI anchored proteins CD55, CD59 on erythrocytes. This disease results from the expansion of clonal hematopoietic stem cells (HSC) which are deficient in GPI anchored proteins Cd55, Cd59 on cell surface as a consequence of inactivation of X linked PIG A gene. The most dominant pathophysiological feature of this disorder is intravascular haemolysis of affected erythrocytes by its own complement system manifested as haemolytic attacks associated with infections [3,17]. Till a decade ago the treatment options were blood transfusion or bone marrow transplantation but since 2007 with the discovery of Eculizumab (ECU) an anti C5 monoclonal treatment of PNH has gained new attention. ECU blocks distal complement pathway and protects red blood cells from complement mediated lysis [3,18].

Apart from disorders caused by genetic mutations in GPI anchor biosynthesis pathway GPI anchored proteins are also involved in many deadly diseases. In following section, some of such diseases are explained giving an emphasis on GPI anchor.

GPI-t subunits and cancer

Cancer is an uncontrolled growth of cells caused by genetic and epigenetic alterations taking place in tumor suppressor genes and oncogenes and results in amplification of oncogenes. GPI anchored proteins play a major role in different types of cancer via the action of enzyme GPI transamidase complex of GPI biosynthesis pathway. These different subunits act as an oncogene and are found to be amplified in many chromosomal regions which are considered as hotspot for cancer. It is more than a decade when PIG-U, one of the subunit of GPI transamidase complex was first identified as an oncogene in bladder cancer cells and since then number of studies are going on to establish the relation between GPI subunits and cancer. Out of five different subunits PIG-K, PIG-S, PIG-T, GPAA1 and PIG-U of GPI transamidase complex, genes encoding PIG-U, PIG-T and GPAA1 are localized in chromosomal region 20q11, 20q13 and 8q24 which are considered as hot spots for cancer [19-21] while genes encoding PIG-S and PIG-T are localized in 1p31 and 17p13 chromosomal region [20,22]. In the following section roles of different subunits of GPI-T are explained.

Amplification of chromosomal region 20q11 is common in many types of cancer including the bladder cancer. The discovery of unique germline translocation on chromosomal region 20q11 in all uroepithelial cancer cells and other cancer cells amplified the significance of this region in malignant cells. A gene encoding CDC91L1 is present on chromosome 20 at position q11 and is present just adjacent to the germ line translocation breakpoint at chromosomal region 20q11 supporting the evidence for its key role as a proto-oncogene in bladder cancer. The genomic amplification and overexpression of this gene is found in human bladder cancer cells and sporadic tumour cells [19,21]. A significant overexpression of PIG-U is also found in breast, lung, and ovarian cancer cells [19,21].

PIG-T is also found in hot spot chromosomal region 20q13.12 [20,21]. PIG-T overexpression was first seen in breast cancer cells and is correlated with downstream overexpression and increased phosphorylation of Paxillin a well-known tumorigenic protein. A significant overexpression of PIG-T is found in colon, thyroid, lung, and pancreatic cancer cell lines [20-22]. PIG-T is also found to be overexpressed in head and neck squamous carcinoma cells. By a combination of mass spectrometry, siRNA inhibition and individual over expression of PIG-T and GPAA1, there are 19 GPI anchored proteins which are identified in breast cancer cell lines but not in healthy breast cells. Overexpression of either PIG-T or GPAA1 affects the cell transduction pathways by increasing the expression of GPI anchored cell surface receptors resulting in increased expression of transcription factor FOXC2, which is overexpressed in breast and colon cancers and is responsible for mitochondrial biogenesis and increased cell metabolism. Cigarette smoke extract also induces the overexpression of PIG-T [20,22].

GPAA1 is another subunit of GPI-T complex and is present in chromosomal region 8q24.3. Significant increase in mRNA levels of GPAA1 are seen in neck and head squamous carcinoma cells and in uterine cancer cells [21-23]. Overexpression of GPAA1 protein is found in ovary and thyroid cells with 40% overexpression seen in prostate cancer and 10-20% overexpression seen in lung adenocarcinoma cells [20-21]. By PCR array profiling of liver tissues

(healthy and tumour samples) out of 117 genes identified whose expressions were altered, GPAA1 was found to be one of those seven genes which was found to be amplified in hepatocellular carcinoma cells and in both hepatocellular B carcinoma positive cells and hepatocellular C carcinoma cells [21,24,25]. Over expression of GPAA1 in cancer cells leads to increase in phosphorylation of Paxicillin by Brk mediating phosphorylation thus promoting cell invasion and mediating tumour metastasis [21,24,25]. CSE also induces GPAA1 by initiating the phosphorylation of Paxicillin in head, neck, bladder, and breast cancer cells. FOXC2 expression is also regulated by the levels of GPAA1 [25].

PIG-K is the catalytic subunit of GPI-T complex and is present on chromosome 1 at position p31.1. Overexpression of PIG-K is seen in uterine and ovarian cancerous cells but it's both m-RNA and protein level is downregulated in bladder, hepatocellular carcinoma cells and in colon carcinoma cells [21]. PIG-K mRNA is also downregulated in prostate and breast cancer cells [21]. PIG-K is found to be accumulated in lymph node cancer samples [20,21].

PIG-S is present on chromosome 17 at position p13.2. Although this gene is overexpressed in breast cancer cells a significant overexpression of PIG-S was seen in thyroid cancer cells. A significant increase in copy number was seen in lung, ovarian, liver, and thyroid cancerous cells [20,21].

Role of GPI anchored protein in cancer

GPI anchored proteins, are of great medical relevance because some specific GPI anchored proteins play a crucial role in tumorigenesis and act as good target for chemotherapy to overcome the challenge faced by biologists in using GPI-T as a chemotherapy agent. Alteration in expression of GPI anchored proteins help in development of new potential drug target. The following section discusses the significance of GPI anchored proteins in cancer along with their relevance as therapeutic target.

Glypican3 is a member of Glypicans which are heparin sulphate proteoglycans and are attached to membrane with the help of GPI anchor. Loss of function mutation in Glypican3 causes a rare x-linked disorder Simpson-Golabi-Behmel Syndrome (SGBS). Glypican3 regulates the activity of several growth factors with the help of their signalling receptors including Wnt signalling. Glypican3 acts as a potential biomarker for hepatocellular carcinoma (HCC) and promotes the progression of the disease with the help of Wnt signalling. Differential expression level of GPC3 on different cancers make it a possible drug target. High expression level of Glypican3 in normal versus malignant liver cells make it a potential target for anticancer activity in liver cells using cell and monoclonal antibody based immunotherapy [26-28].

Urokinase plasminogen activated receptor (uPAR) is a 60 KD GPI anchored protein and is characterized by the presence of three similar functional domains D1, D2 and D3 all connected by di-sulphide bridges. uPAR regulates proteolytic activity on cell surface and in collaboration with other cell surface receptors regulates cell signalling and another gene expression in the cell [21,29]. uPAR is a member of lymphocyte antigen (Ly-6) uPAR protein family and is also found in soluble form (SuPAR) which is generated through the release of entire protein moiety from GPI anchor through proteolytic activity.

In comparison to normal cells uPAR is highly expressed in tissues undergoing remodelling, injury/wound healing and immune response. uPAR is constitutively expressed in majority of cancers including solid tumours, lymphomas and leukaemia and in tumour associated stromal cells such as fibroblasts and macrophages. Binding of uPA to uPAR activates the signalling of protease cascade resulting in degradation of extracellular matrix and tumour cell invasion. Expression level of different components of uPA system act as a biomarker for different cancers and the nature of uPA as a membrane receptor make it a good drug target. Blockage in function of uPA can lead to restricted expression levels of uPA and suppress the tumorigenicity. Most effective way of doing this is by blocking the interaction between uPA and uPAR. Earlier this was done with the help of peptides but now strategies are being developed for targeting the interaction between uPAR and its receptor [29,30].

Prostatin is another GPI anchored protein which maintains salt and fluid balance in kidney by activating sodium epithelial channels. It is overexpressed in pancreatic, breast and ovarian cancer cells. Recently prostatin has also been identified as a tumour marker for early stage of ovarian cancer [21].

Role of GPI anchored protein in neurodegenerative disease: Prion

Prion disease belongs to a family of fatal neurodegenerative disease characterized by spongiform degeneration of

brain and neurological dysfunction. It affects both humans and other animals and its most common form found in humans is Creutzfeldt-Jacob disease (CJD) and scrapie and bovine spongiform encephalopathy (mad cow disease) in other animals [31]. Prion disease is caused by conversion of normal cellular host protein PrP^c (cellular prion proteins) into misfolded, protease resistant pathogenic isoform PrP^{sc} which accumulates within the brain causing neurodegeneration [32]. PrP (Prion protein) is a GPI anchored protein and modifications in the signal sequence of GPI anchor play a critical role in the pathophysiology of deadly disease. The conversion of PrP^c to PrP^{sc} takes place in endocytic compartments or lipid rafts provided that both are attached at the same domain. Any perturbation in lipid rafts like depletion of cholesterol either by inhibiting its biosynthesis or squeezing it by the addition of Fillipin inhibits the conversion of PrP^c to PrP^{sc} and affects the prion conversion. Thus, the presence of PrP^c at lipid rafts becomes a major requirement for conversion of PrP^c to PrP^{sc}. Expression of GPI anchorless PrP^c also abolishes the infection. Prion infection can also be modified by altering the composition and structure of GPI anchor. Exchanging amino acids at C terminal moiety within the SS of GPI anchor of murine PrP^c with rabbit PrP^c also resists the infection. Modification in lipid part part of GPI anchor or of the sialic acid itself alters the cholesterol at the membrane and affects the conversion of PrP^c to PrP^{sc} and hence the infectivity [32,33]. PrP is also found to be upregulated in many cancer cell lines and tumour tissues [34].

CONCLUSION

This review gives a complete view of clinical significance of GPI anchor biosynthesis pathway and GPI anchored proteins in mammalian system. Using a combination of autozygosity mapping and exome sequencing, the study gives information about all IGDs caused by mutations in different genes of GPI biosynthesis pathway in mammals but still many GPI deficiencies are not studied and with the advancement of exome sequencing more of information about these genetic diseases will become possible which will help in better treatment measures. The study also explains how different subunits of GPI transamidase complex are involved in different types of cancer. These subunits have hallmark of oncogene, tumour biomarkers and are overexpressed in different types of cancers and thus act as good target for chemotherapy. The overexpression level of different subunits varies in different types of cancer but the expression level of PIG- K subunit is under expressed and thus become a question of concern that how the decreased expression of one subunit can promote tumour formation? GPI anchored proteins are also found to play a significant role in neurodegenerative Prion disease and the advancement in knowledge of GPI anchor can help in getting better treatment measures for this deadly disease.

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