

## Gene Section

### Review

# TPBG (trophoblast glycoprotein)

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## Abstract

Review on TPBG, with data on DNA/RNA, on the protein encoded and where the gene is implicated.

## Identity

**Other names:** 5T4, 5T4AG, M6P1

**HGNC (Hugo):** TPBG

**Location:** 6q14.1

**Local order**

Size: 7623 bp; orientation: plus strand.

## DNA/RNA

### Description

The gene has 8 distinct introns.

### Transcription

Transcription results in the production of 9 different mRNA, 8 alternatively spliced forms and 1 unspliced form.

The mRNA differ by truncation of the 5 prime end, presence or absence of 2 cassette exons, overlapping exons with different boundaries, splicing versus retention of 3 introns.

The mRNA aAug10 variant has 2590 bp and the premessenger has a single exon. bAug10 has 3395 bp and the premessenger has 3 exons, cAug10 has 3446 bp and the premessenger has 2 exons, dAug10

has 817 bp and the premessenger has 3 exons, eAug10 has 687 bp and the premessenger has 3 exons, fAug10 has 607 bp and the premessenger has 3 exons, gAug10 has 591 bp and the premessenger has 2 exons, hAug10 has 564 bp and the premessenger has 2 exons, iAug10 has 568 bp and the premessenger has 2 exons.

The gene has 3 transcripts or 3 splice variants as per Ensembl. These 3 variants are subsets of the 8 spliced variants as per GenBank, bdEST, Trace and SRA databases supervised by the AceView program.

## Protein

### Note

There is evidence at the protein level.

### Description

This is a 420-amino acid protein that contains an N-terminal putative signal sequence, a 310-residue extracellular region, a membrane anchorage domain, and a 44-amino acid cytoplasmic tail with a potential phosphorylation site. The extracellular region has 7 potential N-glycosylation sites and 7 leucine-rich repeats, which are located in 2 regions separated by a hydrophilic stretch (Myers et al., 1994).

Genomic sequence analysis reveals that the gene has 2 exons, the second of which encodes the protein (King et al., 1999).

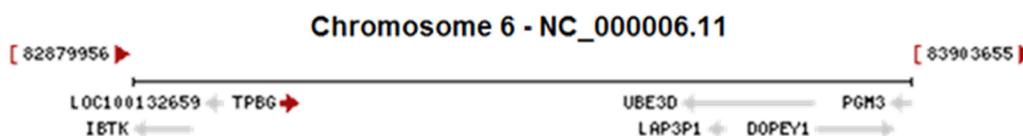


Figure 1. Adapted from Ensembl.

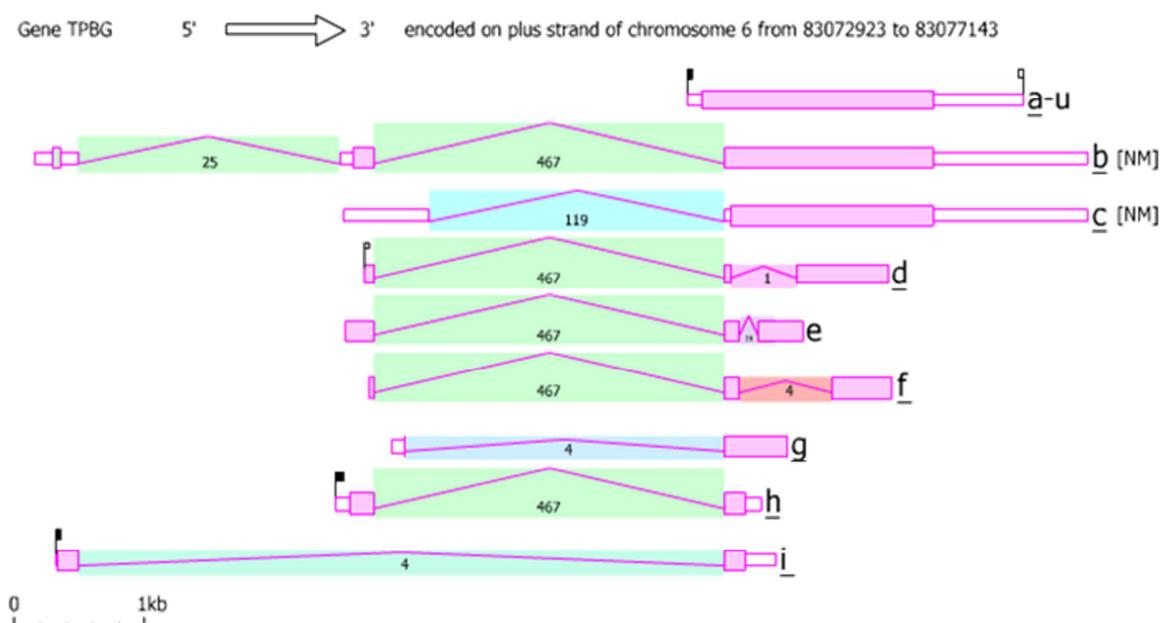


Figure 2. Adapted from NCBI.

### Sequence

The sequence has 420 amino acids and a molecular weight of 46032 (UniProt).

### Isoforms

There are 9 different isoforms (NCBI AceView).

Of the 9 different isoforms, 6 are considered to be very good quality proteins and 3 are good quality proteins.

The 9 isoforms are:

- aAug 10, predicted protein is 593 aa long and 65,1 kDa, and it has one leucine rich repeat N terminal domain, 3 leucine rich repeat domains, one leucine rich repeat C terminal domain and a transmembrane domain;
- bAug10, predicted protein is 588 aa long and 64,3 kDa and it has one leucine rich repeat N terminal domain, 3 leucine rich repeat domains, one leucine rich repeat C terminal domain and a transmembrane domain;
- cAug10 518 aa and 56,6 kDa and has one leucine rich repeat N terminal domain, 3 leucine rich repeat domains, one leucine rich repeat C terminal domain and a transmembrane domain;
- dAug10 272 aa and 30,2 kDa and contains 3 leucine rich repeat domains;
- eAug10 229 aa and 23 kDa and contains no protein domain;
- faug10 201 aa and 22,6 kDa and has 2 leucine rich repeat domains;
- gAug10 161 aa and 17 kDa and contains no protein domain;
- hAug10 117 aa and 13,2 kDa and contains no protein domain;
- iAug10 105aa and 11,9 kDa and contains no protein domain.

There are 2 phosphorylation sites that have been identified based on information summarized in Phosphosite Plus. The first is threonine 99 which was identified by mass spectrometry in the HeLa cervical cancer cell lines (Chen et al., 2009).

The second site of phosphorylation is Serine 418 identified via mass spectrometry in cervical cancer and identified in HeLa cervical cancer cell lines (Olsen et al., 2010).

### Expression

TPBG is expressed by all types of trophoblasts by as early as 9 weeks of development. The gene is specific for trophoblastic cells except for amniotic epithelium. This has been demonstrated by immunoperoxidase staining of frozen sections.

Expression is limited to few epithelial subtypes in adult tissue but is found to be widely expressed on a number of different carcinomas (Southall et al., 1990).

It has a molecular weight of 72 kD.

It is a glycoprotein with a 42-kDa core protein with extensive N-linked glycosylation.

It exists on the cell surface as a monomeric protein. The mature protein is membrane bound and consists of 310 amino acid extracellular regions with 7 N-linked glycosylation sites and a short 44-amino acid cytoplasmic tails.

There are 7 leucine rich repeats in the extracellular portion.

The LRRs are believed to mediate protein-protein interactions (Carsberg et al., 1995).

### Localisation

Membrane, single-pass type I membrane protein.

Tissue	Result	Description
Placenta	+++	Villous trophoblast and amnion
Brain	-	
Ovary	-	
Testis	-	
Skeletal muscle	-	
Heart	-	
Lung	-	
Bronchus	-	
Liver	-	
Spleen	-	
Kidney	+	Glomeruli
Bladder	+	Epithelium
Small intestine	+	Villous epithelium
Uterus	+	Endometrial glands
Cervix	+	Endocervical glands
Skin	+	Basal epidermis

**Table 1.** Reactivity of monoclonal antibody 5T4 with normal human tissues. Immunohistological analysis of frozen sections. Adapted from Hole and Stern, Br. J. Cancer (1988).

## Function

### - Cell adhesion

Transduction into cell lines enhances cell motility and decreases cell-to-cell contact, thereby playing a role in tumor invasion and metastases. This has been demonstrated in mouse fibroblasts (Carsberg et al., 1996).

### - Chemotaxis

It has also been demonstrated that CXCR4-mediated chemotaxis is regulated by 5T4 as shown in mouse embryonic cells. CXCR4 mediates the migration of cells to tissues rich in CXCL12 (Southgate et al., 2010).

### - EMT

The 5T4 antigen has been shown to play an important role in the epithelial-to-mesenchymal transition. The EMT is a process that occurs in early embryonic cells as well as metastases of certain cancers.

The main events that occur during this process are a switch from E cadherin to N cadherin, increased vimentin expression, up-regulation of E cadherin repressor molecules such as snail and slug proteins, increased matrix metalloproteinases such as MMP2 and MMP9, and cellular motility.

The role of 5T4 in the process of EMT has been demonstrated in experiments on embryonic stem cells (Ensembl).

### - Pathways and interaction

There is 1 interacting protein for TPBG-GIPC1 (MINT).

Full expression of 5T4 is found to transform mouse mammary epithelial cells to dendritic morphology. This is accompanied by loss of actin/adherin junctions, down regulation of ecadherin and changes in the cytoskeleton.

These changes do not occur in the absence of the cytoplasmic tail portion of 5T4.

GIPC is a scaffolding protein that interacts with the cytoplasmic tail of 5T4 and it contains the PDZ protein (Lee et al., 2008). PDZ domains mediate protein-protein interactions.

The interaction between GIPC1 and 5T4 was demonstrated in HeLa cells using the yeast two hybrid approach by Awan et al. (2002).

This interaction indicates a role for 5T4 in mediating changes within the cytoskeleton and thereby it's role in invasion and metastases.

## Homology

Orthologs are present from 14 different species with the homology varying from 22% to 99%

The greatest homology was with Pan troglodytes species (chimpanzee) with 99,68% homology based on the nucleic acids and 99,52% homology based on amino acids comparison to the human gene (Ensembl).

There is 1 paralogue (Ensembl).

## Mutations

### Note

**Summary of gene variation consequences** (Ensembl)

There are a total of 921 variant sequences and 33 structural variations as summarized by the Ensembl data base.

This includes 6 frameshift mutations, 6 stop gained mutations, 3 inframe deletions, 153 missense variants, 2 splice region variants, 111 synonymous variants, 26 - 5 prime UTR variants, 117 - 3 prime UTR variants, 22 intron variants, 179 upstream gene variants and 298 downstream gene variants.

Structural variations: there are 12 copy number variations, 2 insertions, 2 inversions, 4 tandem duplications and 13 intrachromosomal breakpoints.

## Implicated in

### Gastric carcinoma

#### Note

5T4 antigen is expressed in up to 81% of gastric cancers. Starzynska et al. (1992) reported that the expression of 5T4 antigen in gastric cancer correlated with the presence of nodal involvement and distant metastases. Review of pathology showed a greater association of the 5T4 antigen with the diffuse variant of gastric cancer as opposed to the intestinal type or mixed pathology.

### Colorectal carcinoma

#### Note

5T4 antigen is expressed in up to 85% of colorectal cancers. Its presence is associated with poor prognosis. The antigen is found more commonly in tumors that present with nodal involvement or distant metastases. This has been demonstrated by IHC staining for the 5T4 antigen. According to a paper published by Mulder et al. (1997), the 5-year survival for tumors that were 5T4 positive was 22%, compared to 75% for tumors that were 5T4 negative. Median survival was 24 months for 5T4-positive tumors, compared to >90 months for 5T4 negative tumors. This indicates that 5T4 antigen expression can be used as an indicator of aggressive disease with earlier recurrence and suggests potential benefit from adjuvant chemotherapy as opposed to 5T4-negative tumors.

#### Drugs and compounds: TroVax

The first study of TroVax in colorectal cancer was in patients who had been previously treated with chemotherapy. In this dose-escalation study, TroVax was given to 22 colorectal cancer patients in an open-label phase I/II trial. Sixteen of 17 immunologically evaluable patients showed a 5T4-specific response. Fourteen patients demonstrated detectable antibody levels following vaccination. There was a positive association between the development of the antibody response and patient survival or time to disease progression (Mulryan et al., 2002).

TroVax has also been studied in combination with systemic chemotherapy in colorectal cancer patients. Two phase 2 studies have studied this combination. In the first study, 12 patients received a combination of 5T4 with 5-FU, folinic acid, and irinotecan. Six had a partial response, and 5 had stable disease. Ten patients had 5T4-specific antibody responses (Harrop et al., 2007).

The second study had a total of 11 patients who received TroVax in combination with 5-FU/folinic acid and irinotecan. Six patients had either a complete response or stable disease, and 10 patients had 5-T4-specific antibody responses (Harrop et al., 2008).

TroVax has been studied as a single agent in patients scheduled for surgical resection of colon cancer. Sixteen patients were enrolled in this phase 2 study. They received 2 vaccinations prior to surgery and 2 vaccinations after surgery. Nine patients had no disease recurrence at 8.4 months, and 13 patients mounted a 5T4-specific antibody response (Elkord et al., 2008).

### Renal cell carcinoma

#### Note

Griffiths et al. (2005) demonstrated the expression of 5T4 antigen in high levels in 20 cases of RCC. The sample was too small to make any conclusions regarding prognosis.

The expression of 5T4 is on the membrane, making it a good candidate for targeted therapy in RCC. Phase 1 and 2 studies have demonstrated the safety and effectiveness of the TroVax vaccine in RCC. The TroVax vaccine is MVA-5T4, which is a modified virus carrying the 5T4 antigen and capable of eliciting an anti-5T4 antibody response.

#### Drugs and compounds: TroVax

Open-label phase 1/2 trial of TroVax administration along with interferon alpha in 11 patients with metastatic RCC. All 11 patients mounted an antibody response to 5T4.

The median time to progression was 9 months which was longer than that with interferon alone (Hawkins et al., 2009).

A second open-label, phase 2 trial involved 23 metastatic RCC patients. TroVax was administered alone or in combination with IFN-alpha. In that study, 96% of patients mounted a 5T4-specific antibody response. One patient in the TroVax/IFN arm achieved a PR; 7 patients in the TroVax/interferon combination arm and 7 patients in the TroVax-alone arm achieved stable disease. The median PFS was 3.8 months, and the median OS was 12.1 months (Amato et al., 2009).

Another phase 2 trial looked at the combination of TroVax with low-dose IL-2 in metastatic RCC patients. Twenty-five patients were enrolled in that study, 21 of whom mounted 5T4-specific antibody response. Two patients demonstrated a complete response of >36 months, 1 patient demonstrated a PR of 12 months, and 6 patients demonstrated stable disease for between 6 and 21 months. Median PFS was 3.37 months, and median OS was 12.87 months (Amato et al., 2008b).

There has been only 1 phase 3 trial to date that has studied TroVax in metastatic RCC patients. Amato et al. (2010) enrolled 733 patients in a study to compare TroVax with sunitinib, IFN, or IL-2. Immune response was assessed in 590 patients, 56% of whom had a positive 5T4-specific antibody response. A high 5T4 antibody response was associated with longer survival in the TroVax arm.

## Cervical carcinoma

### Note

It has been demonstrated that there is a relationship between the expression of 5T4 antigen and dysplastic conditions such as cervical intraepithelial neoplasia (CIN).

Jones et al. (1990) showed a higher level of 5T4 expression in CIN grade 2 and 3 as well as invasive cervical cancer.

It has been postulated that the expression of 5T4 may be directly related to the presence of the human papillomavirus. Jones et al. (1990) studied a total of 66 samples and demonstrated 100% expression of 5T4 in invasive cervical carcinoma. This indicates that 5T4 can be used as a potential tumor marker in cervical cancer.

## Non-small cell lung carcinoma

### Note

Damelin et al. (2011) demonstrated that 5T4 is expressed in tumor-initiating cells and is associated with a poor prognosis in NSCLC. Expression of 5T4 correlated with more undifferentiated histology, shorter time to recurrence, and worse overall survival. The expression of 5T4 correlated with markers of EMT. There were a total of 320 tumor samples in this study, 249 of which were positive for the expression of 5T4 antigen.

## Ovarian cancer

### Note

Wrigley et al. (1995) demonstrated that 71% of epithelial ovarian carcinomas expressed the 5T4 antigen (total sample of 72 tumors). There was a significant correlation between the expression of 5T4 antigen and advanced stage (i.e., FIGO stage 3 and 4) and an association with poorly differentiated tumors. Patients whose tumors expressed 5T4 had a worse overall survival, had shorter disease-free survival, and were less likely to respond to adjuvant therapy.

## Prostate cancer

### Note

#### Drugs and compounds: TroVax

Two phase 2 trials have studied TroVax in castration-resistant prostate cancer patients.

An open-label phase 2 trial by evaluated TroVax alone or in combination with GM-CSF in 27 patients with castration-resistant prostate cancer. All 24 immunologically evaluable patients mounted a 5T4 antibody response. Time to progression was significantly greater in those who mounted a 5T4-specific cellular response (5.6 vs. 2.3 months) (Amato et al., 2008a).

A second phase 2 randomized study enrolled 25 patients, 12 of whom were randomized to receive TroVax plus docetaxel and 13 to receive docetaxel alone. Patients treated with TroVax plus docetaxel

had a longer PFS (9.67 months) than those in the docetaxel-alone arm (5.10 months). Six of the 10 immunologically evaluable patients mounted a 5T4 antibody response (Harrop et al., 2013).

## Other diseases

### Note

Association with other diseases based on cell line studies: GEO Profiles.

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