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Review

Transglutaminase-4 (Prostate Transglutaminase), a Potential Biological Factor and Clinical Indicator for the Diagnosis and Prognosis of Prostate Cancer

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Abstract. *Transglutaminase-4, also known as prostate transglutaminase, is a protein encoded by the TGM4 gene. TGase-4 was thought to be exclusively expressed in the prostate gland and has been suggested to be involved in certain medical conditions, such as infertility and possibly prostate cancer. In recent years, substantial progress has been made in the understanding of this unique protein in prostate cancer, with emerging clinical evidence. The present concise review summarised the current understanding of this intriguing enzyme in prostate cancer and presents an argument that TGase-4 is a useful indicator of both the development and progression of the disease.*

Human prostate transglutaminase, also known as transglutaminase-4 (TGM4), transglutaminase-P (TGP), protein-glutamine gamma-glutamyltransferase 4, and fibrinoligase, is a protein encoded by the *TGM4* gene, located in chromosome 3 (chr3:44,874,608-44,914,990) (1, 2). The *TGM4* gene transcript codes a protein of 684 amino acids and was shown to be primarily located in prostate tissues and is responsive to androgen (3). Although early work had been focused on male infertility, the unique tissue distribution pattern and its portraited biological functions have led to more extensive research into its role in prostate cancer.

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Expression Profile of *TGase-4* in Human Tissues

Although TGase-4 was demonstrated in early days to be almost exclusively expressed in the prostate gland, recent available resources using a wider human tissue profile and new technologies have largely shown that this conclusion does indeed stand. Figure 1A shows the levels of TGase-4 RNA (*TGM4* gene transcript) across a rather large number of human normal tissues which was obtained from the GTEx Portal (accessed on 11th October 2022). It is clear from this comprehensive dataset that the *TGM4* transcript is present at very high levels in the prostate gland, followed by the testis, although by a large margin. It is interesting that both tissues/organs are of the male reproductive system. A much lower expression has been observed in the skin, fallopian tubes, adrenal gland, intestine, urinary bladder, vagina, and lungs, with no difference between males and females, except in the fallopian tubes and vagina.

In human cell lines, the expression varies widely across different cell types from different tissues/organs (Figure 1B). It is interesting to note that cells derived from the bone marrow, proximal digestive system, brain, and female reproductive system tend to have high levels (Figure 1B). In normal prostate tissues, single cell sequencing has shown that TGM4 transcript is largely observed in prostate glandular cells (Figure 1C).

The Biological Impact of *TGase-4* in Prostate Cancer Cells

Like clinical studies, biological-oriented studies on TGase-4 are not extensive. In collaboration with Dr. Richard Ablin of the University of Arizona, we have carried out a number of investigations by creating cell line models from prostate cancer. In this respect, we have generated both over-expression and knockdown models in prostate cancer cells that

normally displayed either low or high expression levels of TGase-4. We have demonstrated in a series of *in vitro* assays, that high levels of TGase-4 in prostate cancer cells rendered them with a high degree of matrix adhesiveness, and increased migration, and invasiveness (4). Increased TGase-4 in prostate cancer cells also increased their degree of adhesiveness to vascular endothelial cells (5, 6). It was also demonstrated that exogenous TGase-4 was also able to induce epithelial-to-mesenchymal transition (EMT), a biological process fundamental to the progression of cancer (7).

Perhaps one of the most interesting findings is that *TGM4* is a highly responsive gene to male hormones (1). Recent findings from a murine-based prostate cancer model by Lopez-Bujanda *et al*. demonstrated that TGM4 is a highly responsive gene to androgen in that it may vary (increase) by a fold of one thousand times along with *MSMb* and *Sink1* (8). TGM4 is also one of the most up-regulated genes in prostate tumours in comparison with normal prostate tissues in the murine model.

TGase-4 in Clinical Prostate Cancer

The clinical association between TGase-4 and prostate cancer has been slowly emerging and is somewhat controversial. In early studies, TGase-4 transcript, by way of Northern blot and PCR, was found to be at low levels in prostate cancer compared with normal tissues (9). This pattern appears to be similar to another member of the transglutaminase family, namely tissue transglutaminase (TGase-2), which showed lower levels in prostate cancer tissues and prostate cancer cells than normal tissue and normal prostate epithelial cells (10). This was also supported by a transcript analysis from laser-dissected prostate tissues in that prostate cancer cells had lower levels of the TGM4 transcript than normal prostate tissues (11). However, more recent studies appear to be in contrast. For example, an immunohistochemistry-based investigation has shown higher levels of TGase-4 protein in human prostate tumours than in associated normal tissues and that this high level is an indicator of a poor biochemical recurrence-free survival (12). We have also shown that in a selective prostate cancer cohort, there were increased levels of TGase-4 transcript in prostate tumours with a Gleason Score higher than 7 (13). From microarray data sets, it was clear that TGase-4, along with a few other gene products, are connected to tumorigenesis in the prostate (14). This has received support from a much larger study from the TCGA dataset as recently reported by Lopez-Bujanda *et al*. (8). The study, again using biochemical recurrence as a clinical indicator, found that high levels of *TGM4* gene transcript is linked to a significantly shorter survival. Amongst the tumour types compared, the degree of *TGM4* transcript rise was also the greatest in prostate cancer compared to other types, followed by leukaemia, squamous cell carcinoma of head and neck, and lung origins.

Using some recent technologies, namely MudPIT on a LTQ-Orbitrap XL mass spectrometer, Kim *et al*. discovered that TGase-4, along with TIMP1, SEFn PARK7, PSA, prostate acidic phosphatase (PAP), and MME, are significantly elevated in the prostatic secretions of patients with primary and recurrent prostate cancers (15). Perhaps the most direct evidence for the link between TGase-4 and prostate cancer within a larger cohort was reported in a recent study by Cao *et al*. (12). Using a clinical cohort of 159 patients with prostate cancer who received radical prostatectomy, the authors carried out immunohistochemical analyses of TGase-4 along with other markers and reported that TGase-4 was highly expressed in prostate tumour tissues compared with non-cancerous tissues. TGase-4 staining was also highly correlated with Gleason Scores and PSA levels, in line with early findings on TGase-4 transcript. One of the most interesting findings was the significant correlation between TGase-4 and the recurrence (biochemical) of prostate cancer, which, along with the Gleason score (but not PSA interestingly), was found to be an independent prognostic factor for the biochemical recurrence (12). However, the controversies remain from early days, including a recent study by Shan *et al*., in which needle biopsies from 105 individuals with 'abnormal' PSA or abnormal findings on digital rectal examination indicated that 57 cases had prostate cancer (PCa) and 48 cases benign prostatic hyperplasia (BPH). A study that employed microarray technologies to identify differentially expressed genes, showed that *TGase-4* expression, along with that of *HOXA7* and *KRT15*, was significantly lower in prostate tissues compared with BPH tissues (16). This recent study is somewhat contradictory to other observations for the following reasons. First, the nature of the tissues. The study used biopsy tissues and did not carry out microdissection. It is likely that the biopsy samples contained a mixture of cell and tissue types including normal epithelial cells, stromal cells, and cells of other types and that different cell types and tissues express varying levels of TGase-4. Second, the study compared BPH and prostate cancer. It would have been ideal to perform additional comparisons and also include normal tissues. Indeed, HOXA7 has been found to be highly expressed in liver cancer (17), ovarian cancer (18), squamous cell carcinoma of the oral cavity (19), and breast cancer (20), and be associated with disease progression in these tumour types. A similar trend for KRT15 (keratin-15) to HOXA7 was reported squamous cell lung carcinoma (21), and colorectal cancer (22). This would suggest that the cohort evaluated by Shan *et al*. should be reexamined or other methods to be used for validation. This possibility is partially validated by a study by Sequeiros *et al*., in which TGase-4 expression was found to be lower in urinary extracellular vesicles from prostate cancer patients compared with that in patients with benign prostate conditions (23).

Thus, although there appears to be a close link between TGase-4 and prostate cancer, a clear conclusion is yet to be

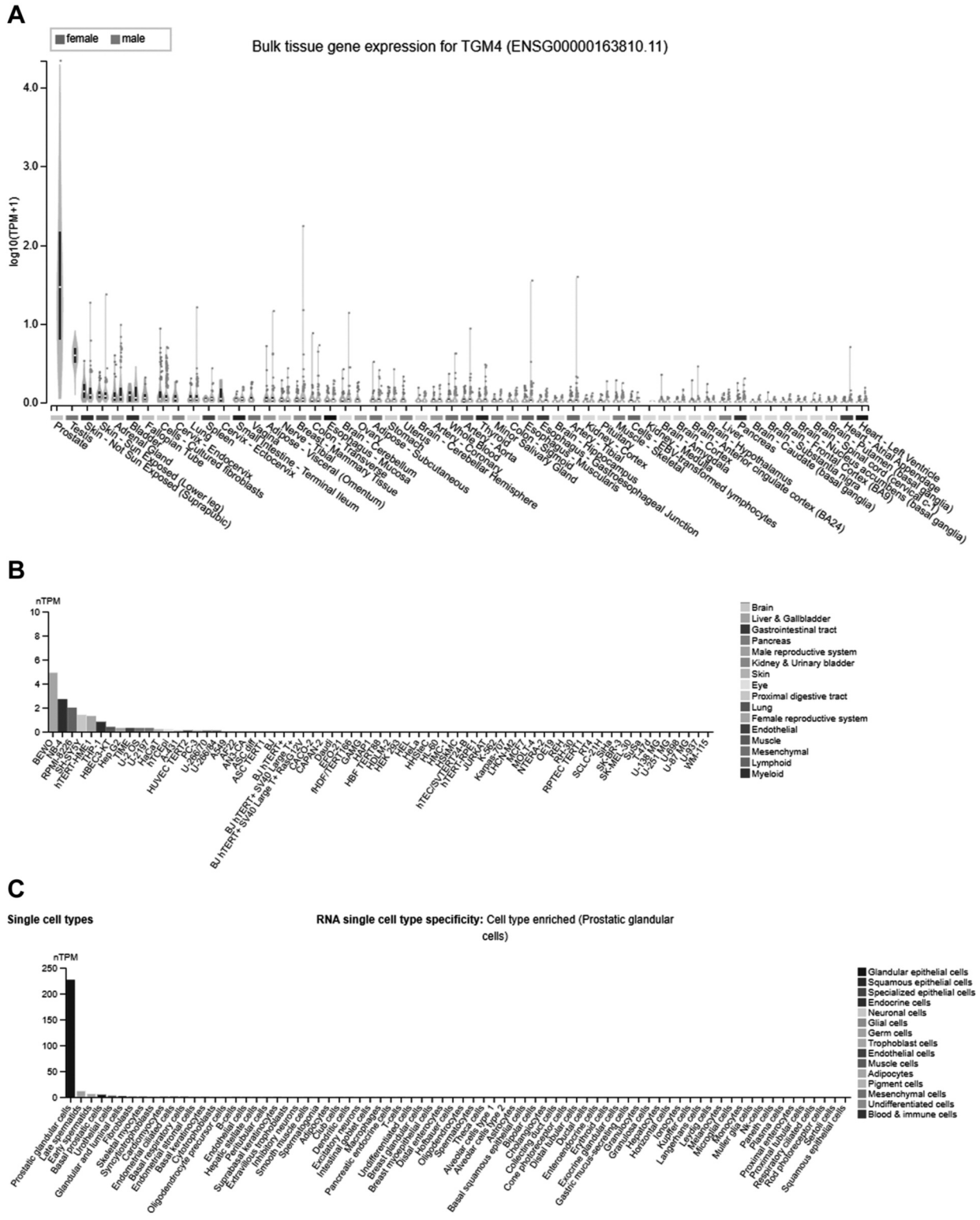

Figure 1. *Expression profile of the TGM4 gene transcript in cells, human tissues, and single cell types in the prostate gland. A) The TGM4 gene transcript in human tissues was obtained from the GTEx Portal (https://gtexportal.org) on 11/10/2022. Shown are tissues from males (left of each tissue type) and females (right of each tissue type). The prostate gland has the highest levels of the gene transcript. B) Expression of the TGM4 gene transcript in human cell lines, dataset from the human protein atlas database (27). C) Presence of the TGM4 transcript in different cell types of the prostate gland, single cell sequence from the human protein atlas (27).*

drawn, largely due to the inconsistency amongst the clinically-oriented investigations. Whilst most studies indicate an over-expression of TGase-4 in prostate cancer when compared with normal and benign tissues, some of the studies have demonstrated otherwise. It was clear that some of the inconsistencies were due to the methodologies and the way that tissues were processed; others could be due to the specific comparisons performed, namely tumour *vs*. BPH *vs*. normal, tissues *vs*. cells, tissues *vs*. extracellular vesicles *etc*. It was also very interesting to observe that the inconsistencies bear similar hallmarks to the other important prostate marker, namely PSA, which the entire literature shows the controversies regarding its expression levels in normal, tumour, and BPH tissues. A recent example is that prostate cancer tissues, in particular those of high grade, had lower levels of PSA than low grade tumours and BPH tissues, which together with other reports indicate that PSA is a measure of the volume of the prostate gland and of prostate tumours rather than a specific tumour marker. TGase-4, with the limited number of available studies in the literature, may have similarities to PSA as a tissue marker. Thus, whilst studies using tissue and immunohistochemical methods appear to deliver more controversial findings, recent *TGM4* gene transcript-based studies appeared to be more consistent, which demonstrated TGM4 as a highly useful prognostic biomarker for patients with prostate cancer. This has shown reproducibly in some other mRNA-based studies, where the levels of the *TGM4* gene transcript were significantly higher in prostate cancer tissues than in benign prostate tissues (24, 25).

Would *TGase-4* Be a Useful Prognostic Serum/Body Fluid Factor for Prostate Cancer and Prostatic Diseases?

Perhaps one of the most interesting and intriguing studies in assessing the validity of TGase-4 as a diagnostic or prognostic factor is a recent investigation by Drabovich *et al*. in which the level of TGase-4 in seminal plasma was determined in a series of patients (26). Significantly higher levels of TGase-4 were seen in patients with prostate cancer than in those with benign prostate conditions. The validation cohort of seminal plasma has returned with a marked increase in the ratio of TGase-4 between patients with prostate cancer (n=152) to those with benign biopsies (n=67) (Ratio 3.1, p=0.00075), making TGase-4 the only marker with a significant difference between the two groups out of nineteen candidate markers and six control markers including KLK3 (PSA) (Ratio 0.81, p=0.11). The study has also shown that seminal plasma and tissue biopsies, but not the serum, are mostly suited for ELISA and mass spectrometry-based selected reaction monitoring.

The comprehensive study by Drabovich *et al*. has also shown that TGase-4 concentration in seminal plasma is more than two thousand times higher than that in blood serum (26). It is interesting to note that the study did not find TGase-4 in blood serum as a useful marker in distinguishing those with prostate cancer from those without, a sharp contrast to TGase-4 in seminal plasma. This convincing study thus suggests that seminal TGase-4 is a potential diagnostic marker for prostate cancer to be detected in seminal fluid or prostate tissue and instead TGase-4 in blood serum may not be a suitable sample type for the test. This may also provide some clue as to why there has been so few studies on TGase-4 in blood serum. The study has also shown that TGase-4 expression in younger males (age <40 years) tends to be significantly higher compared to that in those with higher age, >70 years of age (p<0.01). A similar and striking difference was found for blood serum TGase-4.

Sequeiros *et al*., whilst investigating protein markers in the urinary extracellular vesicle, found that the levels of TGM4 in samples of prostate cancer were lower than those from benign conditions (23).

TGase-4 as a Therapeutic Target

With the evidence supporting an important role of Tgase-4 in prostate cancer, whether TGase-4 may be considered as a therapeutic target has raised interest and is a subject of recent investigations. A recent report by Lopez-Bujanda *et al*. showed that TGase-4 is a potential immunogenic target for prostate cancer and that immune cells treated with TGase-4 can elicit anti-tumour activities *in vitro* (8). The study also found that patients (30%) who were treated with granulocyte-macrophage colony-stimulating factor gene-transduced irradiated prostate cancer vaccine cells (GVAX) responded by producing anti-TGM4 IgG and this immunogenic response appears to be connected with a lower chance of PSA recurrence. These intriguing results will undoubtedly be followed by more intensive investigations.

Perspectives

TGase-4, a protein primarily expressed in the prostate gland, has an important biological impact on prostate cancer cells. Clinically, there is increasing evidence that its presence and levels in seminal plasma and in prostate cancer tissues present a diagnostic and prognostic opportunity in clinical prostate cancer. There remain significant challenges in elucidating the expression pattern of both the protein and gene transcript of TGase-4 in tissues. Whilst it was clear that seminal TGase-4 protein appears to be a good diagnostic factor for prostate cancer, the importance of the protein levels in blood serum and tissues remains elusive and certainly deserves further investigation. Although the biological impact of TGase-4 on prostate cancer has been investigated to some degree, given that the protein can be

readily detected in body fluids, the full profile of the protein in normal prostate epithelial cells and prostate cancer cells needs further in-depth study. Finally, there is exciting evidence to indicate that TGase-4 can be used as a target for immunotherapy. Whether it can also be used as a target for other approaches remains an interesting topic.

Conflicts of Interest

The Authors declare that there are no conflicts of interest in relation to this study.

Authors' Contributions

LY, AS and WGJ drafted and revised the manuscript.

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