International Journal of Allied Medical Sciences and Clinical Research (IJAMSCR)

ISSN:2347-6567

IJAMSCR | Volume 9 | Issue 3 | Jul - Sep - 2021
www.ijamscr.com

Research Study *Medical research*

Alteration Of Hernia Susceptible Genes In Colorectal, Stomach And Testicular Cancers

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ABSTRACT

Background: There are reports indicating the association of colon carcinoma with inguinal hernia. Four novel Hernia susceptible genes have been identified in a genome-wide association study. Evaluating the status of hernia susceptible genes in colorectal, stomach and testicular cancers will provide some insights about the co-occurrences or severity of cancer. Current study was designed to evaluate the expression of hernia susceptible genes and their proteins in colon, stomach and testicular cancers and their respective normal tissues and determine their interacting proteins.

Methods: University of California Santa Cruz (UCSC) Xena browser of TCGA TARGET GTExdatabase was used for determining the mRNA expression of herniasusceptible genes *EFEMP1*, *WT1*, *EBF2* and *ADAMTS6* in primary tumour of colorectal, stomach and testicular cancers and their respective normal tissues. Utilizing the Human Protein Atlas (HPA) database, differences in the expression of proteins encoded by hernia susceptible genes were determined normal tissues and the primary tumours of colon, stomach, and testis. STRING, protein-protein interaction network analysis was used to evaluate interaction protein partners of hernia susceptible genes.

Results: In the colorectal cancerprimary tumour tissue EFEMP1 and EBF2 were significantly downregulated,and WT was significantly upregulated compared to normal colon tissue. There was no change in the expression of ADAMTS6.In stomach cancers mRNA of WT1, EBF2 and ADAMTS6 were significantly upregulated and EFEMP1 was downregulated in primary tumours compared to normal stomach. In testicular cancers, mRNA expression of EFEMP1 was upregulated and WT1, EBF2 and ADAMTS6 were downregulated compared to normal testis.Protein-protein interaction studies revealed that all the four hernia susceptible genes interacted with the proteins associated with tissue remodelling and immune response.

Conclusion: Our results showed that hernia susceptible genes were differentially expressed in colorectal, stomach and testicular cancerssuggesting their functional significance in these cancer patients and some of these may serve as markers in the treatment intervention.

INTRODUCTION

Hernia defined as protrusion of an organ through and abdominal opening in the muscle wall of the cavity is one of the common ailments. More than 20 million herniasare repaired yearly in the world(HerniaSurge 2018). The number of repair surgeries in a year varies in different countries range from 100 to 300/100,000 populations(Kingsnorth and LeBlanc 2003). The annual estimated Inguinal hernia in India is 1,957,850(Rao S S et al. 2016; Primatesta and Goldacre 1996). Hernia may occur due to several reasons including congenital,obesity, muscular weakness, surgery, illness, or failure of close of certain structures after birth(Glanze WD et al. 1986). Abdominal wall hernia is common with a prevalence of 1.7% for all ages and 4% for above 45 years old(Jenkins and O'Dwyer 2008).Hernia is very common in men than women with risk factor of 27% and 3% respectively (Jenkins and O'Dwyer 2008). Recent study revealed lifting of heavy objects as a common cause for the presence of hernia (Bollu J, Sunkara VR , and S 2020; Jha S and D 2020).The genetics associated with hernia is understudied and there are no detailed studies to understand the precise molecular mechanism(s) associated with hernia. One of the comprehensive genome-wide association study identified four novel

susceptible loci in the regions of *EFEMP1*, *WT1*, *EBF2* and *ADAMTS6* underlying in inguinal hernia (Jorgenson et al. 2015). This study highlighted the genetic pathways associated with hernia development and its treatment. Hernias are benign and get cured with hernia repairs without recurrence for groin hernia(Ramjist et al. 2019; Schmidt et al. 2018)but for the ventral hernias the recurrence rates are high (Hajibandeh et al. 2017; Venclauskas et al. 2017). Even though hernia by itself may not be detrimental, but when it encounters with other illnesses especially cancer may aggravate each other and treatment response may be adversely affected(Kulacoglu and Kockerling 2019).

Cancer is one of the leading cause for death in all most all the countries in the world (Bray et al. 2021). The estimated new cancer cases in the world is 19.3 million and almost 10 million cancer deaths occurred in 2020(Sung et al. 2021). The expected global burden of cancer in 2040 will be 28.4 million cases, an increase of 47% from 2020, with larger increase in transitioning countries than transitioned countries. Colorectal and Stomach cancers are 3rdand 4thmost commonly diagnosed cancers in the world with the number of cases of 1,148,515 and 1,089,103 respectively and these cancers remained as 2nd and 4thleading cause of cancer deaths in the world. Although there is no strong association of hernia and cancer, there are publications indicated that the hernia may develop in cancer patients. Inguinal hernias association with colon cancer is known(Birolini et al. 1998; Davis and Jackson 1968; Mizuno et al. 2019; Salemans et al. 2013; Terezis NL., Davis WC., and FC. 1963).but there are no clear indications of cause and effect(Kulacoglu and Kockerling 2019). There is one case report indicating that hernia association with gastric cancer(Mimatsu et al. 2014). There are several reports indicating the correlation between inguinal hernia and testicular cancer. Testicular cancer risk was three times higher in men with inguinal hernia without any association between hernia and tumours (Coldman, Elwood, and Gallagher 1982; Morrison 1976). Furthermore, hernia repair before age of 15 years was found as a risk factor for seminoma (Group 1994; Pottern et al. 1985; Swerdlow, Huttly, and Smith 1987) as well as testicular cancer (Cook et al. 2010; Gallagher et al. 1995; Honecker et al. 2018; Mai et al. 2010; Ondrusova and Ondrus 2009). All these reports indicated the point where the roads of hernia and cancer intersect (Kulacoglu and Kockerling 2019). In terms of treatment strategies, there are no treatment methods to which both cancer and hernia respond well, the common of choice for both is surgical intervention. There is a possibility that treatment for cancer could adversely affect hernia and vice versa(Kulacoglu and Kockerling 2019). Tamoxifen, a hormonal therapy for breast cancer has been showed as a promoter of hernia development in mice by activating the matrix metalloproteinase(Ma et al. 2015). In gynaecological cancer patients accelerated incisional hernia development was observed with radiotherapy and treatment with bevacizumab and liposomal doxorubicin. Bevacizumab, an antiangiogenic agent used in combined with chemotherapy, provided promising results in resistant and metastatic cancers(Collot et al. 2018; Qu et al. 2015; Venook et al. 2017), but increase the incidence of wound healing complications (Zhang et al. 2016) and intra-abdominal issues like intestinal fistula(Eveno et al. 2014; Hapani, Chu, and Wu 2009). Additionally enterocutaneous fistula was showed as an adverse effect of bevacizumab(Eriksen and Bulut 2014). Symptomatic incisional hernia was more common after 1 year in the patients given bevacizumab (Allegra et al. 2009). In another case report study , patient received chemotherapy and bevacizumab for colorectal liver metastasis developed incarcerated recurrent Incisional hernia (Giakoustidis et al. 2014). Rapamycin, immunosuppressant and anti-tumour agent was showed as an inhibitor of vascularization around synthetic mesh(Laschke et al. 2009; Law 2005).All these published reports indicated that there are cancer patients who may encounter hernia and vice versa.

The current study was aimed to investigate the hernia susceptible genes expression in cancers of colorectal, stomach and testis compared to their respective normal tissues. Our results showed the differential expression of hernia susceptible genes *EFEMP1*, *WT1*, *EBF2* and *ADAMTS6*in primary tumour tissues of colorectal, stomach and testicular cancers. These results suggest that hernia susceptible genes functional significance and may be serve as potential markers to evaluate the therapeutic strategies efficacy in cancers where hernia may develop as a co-occurrence.

METHODS

TCGA Target GTEx data

We selected three human cancers primary tissues of colorectal, stomach and testis whose anatomical sites are known as potential sites for hernia development. Corresponding normal tissues were included for gene expression analysis. Tumour adjacent normal tissues and the metastatic tumour tissues were not considered in this analysis. Normalized mRNA expression of four hernia susceptible genes *EFEMP1*, *WT1*, *EBF2* and *ADAMTS6* was downloaded in primary tumour tissues of colorectal, stomach and testicular cancers and their respective normal tissues of colon, stomach and testis from The Cancer Genome Atlas, TCGA TARGET GTEx database(Goldman et al. 2020) using UCSC xenabrowser (https://xenabrowser.net). The gene expression was available on 308 normal colon tissues and 288 colorectal primary tumour tissues. There were 174 normal stomach tissues samples and 414 primary tissue samples from stomach cancer. In the testicular cancer, there were 165 tissues of normal testis and 154 samples of primary testicular tumour tissues. The gene expression was compared between normal and pri5mary tumour tissues in all the three types of cancers. De-identified human samples data from publicly available database was obtained therefore there was no requirement for Institutional Review Board (IRB) approval for these studies.

The Protein Atlas database

The Human Protein Atlas version 20.1 was utilized to evaluate the hernia susceptible genes encoded protein expression by Immunohistochemistry (IHC)(Uhlen et al. 2015). IHC images were availableon the expression of EFEMP1, WT1 and ADAMTS6 in the tissue sectionsof normal colon, stomach, and testis in the Tissue Atlas. These images were downloaded. Corresponding pathology IHC images available in the Pathology Atlas were downloaded on the primary tumour tissues of

colorectal, stomach, and testicular cancers. All the photomicrographs were downloaded at the magnification of 25µm. Antibodies used for IHC studies were validated by standard procedures, high resolution images were generated, and the staining intensity was scored by pathologist.IHC staining intensity was scored in the database as High (H), medium (M), low (L), and non-detected (ND) which was showed in the images. IHC data on EBF2 was not available in the database. The percent tumourand normal tissues stained for intensity as low, medium and not detected was used to graphical presentation to indicate the number of tumours/normal tissues stained for the specific hernia susceptible proteins.

STRING, protein-protein interaction network analysis database

Hernia susceptible genes EFEMP1, WT1, EBF2 and ADAMTS6 were included to search for their protein interactions using the STRING Database version 11.0 (https://string-db.org)(Szklarczyk et al. 2019). STRING database revealed the known and predicted protein-protein interactions. These interactions were based on direct physical interactions, indirect functional association, computational prediction, transfer of knowledge between organisms, and aggregated from other primary databases. Interactions were showed in the form of colour nodes indicated first shell of interaction and white nodes as second shell of interactions. Node content was showed as empty for unknown 3D structure of protein and filled nodes for 3D structure known or predicted proteins. Edges were showed in different colours for known interactions, predicted interactions and others.

Statistical analysis

The gene expression data was analysed for statistical significanceusing *t*-test by selecting Mann-Whitney test and comparison ranks. Analysis results were considered significant if the p-value was < 0.05. GraphPad prism software (version 8.0.2, San Diego, California, USA) was used for the statistical analysis..

RESULTS

Hernia susceptible genes were differentially expressed in colorectal, stomach and testicular cancers

Colorectal cancer

The expression of four hernia susceptible genes *EFEMP1, WT1, EB2* and *ADAMTS6* was evaluated in 288 colorectal cancer primary tumours and 308 normal tissues. The results presented in **Figure 1** revealed that WT was significantly upregulated in primary tumours. *EFEMP1* and *EBF2* were significantly downregulated and there was no change in *ADAMTS6*. These results suggest that the increased expression of *WT1* in colorectal cancers may be associated with the co-incidence of hernia in colorectal cancer patients.

Stomach Cancer

Messenger RNA expression data from 413 stomach primary tumour tissues and 173 normal tissues was retrieved from TCGA Target GTEx database and analysed for the differential expression of four hernia susceptible genes. 3 out of 4 (75%) hernia susceptible genes (*WT1,EBF2* and *ADAMTS6*) were significantly upregulated in stomach primary tumours compared to normal stomach. Furthermore, EFEMP1 was significantly downregulated in the primary tumours. (**Figure 2**). Overall, all the four hernia susceptible genes were regulated in stomach primary tumour tissues compared to normal stomach tissue, indicated the possible co-occurrence of hernia in patients with stomach cancer.

Testicular Cancer

Since the occurrence of hernia has been noted in testicular cancer patients, we have evaluated the expression of *EFEMP1*, *WT1*, *EBF2* and *ADAMTS6* in 153 testicular cancer primary tumour and 164 normal testis tissues utilizing the TCGA Target GTExdatabase. Like stomach cancers, testicular cancers also exhibited all the four hernia susceptible genes alteration in primary tumour tissues compared to normal testis. Among the four hernia susceptible genes *EFEMP1*was the only one gene significantly upregulated in primary tumours compared to normal testis. The other three genes *WT1*, *EBF2* and *ADAMTS6* were significantly downregulated in testicular primary tumour tissues (**Figure 3**). These results indicate that EFEMP1 may be associated with the occurrence of testicular cancer in hernia patients or vice versa. Additional studies are needed to identify specific functions of these genes in hernia development in the co-occurrence of hernia in testicular cancer patients.

Hernia susceptible genes encoded proteins were differentially expressed in colorectal, stomach and testicular cancers

Colorectal cancer

To evaluate whether the proteins encoded by hernia susceptible genes were differentially expressed in the primary tumour tissues of colorectal, stomach and testicular cancers compared to their respective normal tissues, we have used the publicly available database in the Human Protein Atlas (HPA). HPA database contain IHC proteins expression data of three hernia susceptible genes EFEMP1, WT1 and ADAMTS6. IHC data on WT1 was not available in HPA database. There were 12 colorectal primary tumour tissues and 3 normal tissues included in the IHC data. EFEMP1 was not detected in 100% (12 out of 12) primary tumours, whereas in normal tissues, the expression was low 100% (3 out of 3)in all the tissues (**Figure 4 upper panel**). WT1 was detected as low in 3 out of 3 (100%) in normal colon, in the tumours it was detected low in 1 out of 12(8%) and not detected in 11 out of 12 (92%) (**Figure 4 middle panel**). ADAMTS6 was found at medium level in 3 out 3(100%) normal tissues, where as in tumour tissues it was not detected in all the 12 (100%) tissues (**Figure 4 bottom panel**). EFEMP protein expression data agrees with mRNA data being low in tumours compared to normal tissues. Protein expression data of WT1 and ADAMTS6 was not agreed with the mRNA expression data.

Stomach Cancer

HPA contained expression data on 12 stomach cancer tissues and 3 normal stomach tissues. Expression of hernia susceptible protein EFEMP1 was slightly higher in 1 out of 12 (8%) primary tumours and there was no detectable EFEMP1 in 3 out 3 (100%) normal (**Figure 5**

upper panel). The mRNA expression of EFEMP1 was significantly low in primary tumours of stomach compared to normal (**Figure 2**). Like EFEMP1, WT1 protein was also slightly increased in 1 out of 12 (8%) primary tumours and not detected in 3 out of 3 (100%) normal tissues. (**Figure 5 middle panel**). ADAMTS6 was detected in both normal stomach and stomach cancer primary tumours (**Figure 5 bottom panel**).

Testicular Cancer

There were 12 testicular primary tumours and 3 normal testis tissues available in HPA database. EFEMP was found increased in 25% (3 out of 12) primary tumours compared to not detected in normal testis tissues (**Figure 6 upper panel**). Both WT1 and ADAMTS6 were not detected in either normal testis or primary tumour tissues of testicular cancers (**Figure 6 middle and bottom panels**)

Hernia susceptible proteins interact with tissue remodelling and immune response proteins

To identify the interacting protein partners of hernia susceptible proteins, we have utilized STRING database, a Protein-Protein interaction networks functional enrichment analysis. The data presented in **Figure 7**showed the different proteins interact with each of hernia susceptible proteins. EFEMP1 interact with four different proteins microfibrillar-associated proteins (MFAP1,2,3,4,5); Fibulin (FBLN1,2,5); Vitronectin (VTN), and EGF-containing fibulin-like extracellular matrix protein 2 (EFEMP2) (**Figure 7A**). WT1 interact with eight different proteins including paired box protein Pax2 (PAX2), tumour protein p73 (TP73), telomerase reverse transcriptase (TERT), DnaJ homolog subfamily A member 3 (DNAJA3), Cellular tumour antigen p53 (TP53), Nephrin (NPHS1), Cbp/p300-interacting transactivator 2 (CITED2), Transcription factor SOX-9 (SOX9), Transcription factor 21 (TCF21), Protein Wnt-4 (WNT4) (**Figure 7B**). EBF2 interacted with 10 proteins including PR domain zinc finger protein 16 (PRDM16), Transmembrane and tetratricopeptide repeat containing 1 (TMTC1), Zinc finger protein 423 (ZNF423), Krueppel like factor 11 (KLF11), Guanine nucleotide binding protein (Golf) subunit alpha (GNAL), Sideroflexin-4 (SFXN4), Mitochondrial brown fat uncoupling protein 1 (UCP1), Transducin-like enhancer protein 3 (TLE3), Cold shock domain containing protein E1 (CSDE1), and Cell death activator CIDE-A (CIDEA) (**Figure 7C**). ADAMTS6 found to interact its owns isoforms and two other proteins. The results presented in **Figure 7D** showed the interacting proteins GDP-fucose protein D-fucosyltransferase 2 (POFUT2), A disintegrin and metalloproteinase with thrombospondin motifs 13 (ADAMTS13), ADAMTS-like protein 2 (ADAMSL2), ADAMTS-like protein 2 (ADAMTSL2), ADAMTS-like protein 1 (ADAMTSL1), ADAMTS-like protein 4 (ADAMTSL4), Thrombospondin 1 (THBS1), A disintegrin and metalloproteinase with thrombospondin motifs 1 (ADAMTS1), A disintegrin and metalloproteinase with thrombospondin motifs 3 (ADAMTS3), ADAM metallopeptidase with thrombospondin motifs 17 (ADAMTS17), A disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5).

DISCUSSION

The roadmap of hernia and cancer has been reported indicating certain intersection points of these diseases are interested to further research. Though there is no evidence in the common etiology for hernia and cancer, these two different diseases had some common points on biomarkers, genes and integrins(Kulacoglu and Kockerling 2019). There are no reported studies on the status of hernia susceptible genes expression in the cancers and their normal tissues of colorectal, stomach and testis where hernia may occur. In this study we have selected four hernia susceptible genes Epithelial growth factor-containing fibulin-like extracellular matrix protein1 (*EFEMP1)*, Wilm's tumour (*WT1)*, *EBF2* and *ADAMTS6*(Jorgenson et al. 2015)to evaluate their expression in cancers occurring in colorectal, stomach and testis where hernia may occur. All these four genes associated with direct or indirect inguinal hernia were found to express in mouse connective tissue, EFEMP1 was found highest. Connective tissue maintenance and homeostasis have been associated withEFEMP1 and WT1 with their action on extra cellular matrix ECM enzymes such as matrix metalloproteases which are involved in the degradation of collagen and elastin (Jorgenson et al. 2015). Deregulation of collagen homeostasis has been demonstrated as one of the key factors in the development of inguinal hernia(Bendavid 2004). Collagen is the main structural protein and undergoes continuous synthesis and degradation process (Antoniou, Georgiadis, et al. 2011), found to be lower in hernia patients samples along with the ratio of type I to type III(Casanova, Trindade, and Trindade 2009). Furthermore, it was found that the imbalance of collagen degrading matrix metalloproteinase and their inhibitors (MMPS and TIMPs) in hernia patients (Antoniou, Tentes, et al. 2011) supports the functional significance of EFEMP1 and WT in hernia development. It is known that WT1 function as inhibitor of MMP2(Rung et al. 2006) and activator of TIMP3 (Baudry et al. 2002) that leads to the inhibition of MMPs. Additionally, EFEMP1 interact with TIMP3 that may increase the inhibitory role of WT1 on MMPs(Klenotic et al. 2004). ADAMTS belongs to the matrix metalloproteinase family which are involved in the conversion of procollagen to collagen (Brocker, Vasiliou, and Nebert 2009). We found deregulation of all four genes whose functional significance is known in the development of hernia in colon, stomach, and testicular cancers (**Figures 1 to 6**) suggest that these cancer patients may be more susceptible to hernia development if they encounter the hernia risk factors.

Recent studies on demographic and risk factors associated with hernia in a tertiary hospital revealed that the rural area illiterate occupation population was affected more by hernia and weight lifting was showed as a risk factor (Bollu J, Sunkara VR , and S 2020).Cancer incidence, mortality and survival disparity in Rural-Urban areas is known (Li et al. 2018; Rovito et al. 2020; Zahnd et al. 2019; Zahnd et al. 2018). Malignancies present in the hernia originating organs such as colorectal, stomach and testis may be happening higher in rural area population especially in underdeveloped areas. Evaluating the hernia susceptible genes in these cancers may provide some clues to better understand the molecular alterations in hernia and cancer which may

serve as biomarkers. There are published data indicating certain chemo and radiotherapy modalities may cause herniation with an effect on tissue repair. Bevacizumab, an anti-angiogenic agent widely used for cancer treatment may cause serious complications and interfere with hernia repair(Jorgenson et al. 2015).Here we attempted to evaluate the expression levels of hernia susceptible genes and their encoded proteins in normal and primary tumour tissues of colorectal, stomach and testicular cancers. We have some limitation on protein expression data with low number of primary tumour tissue samples (n=11 to 12) and normal tissues (n=3) in HPA database. TCGA database contain mRNA gene expression data on 308 normal colon and 288primary tumours of colorectal cancer, 174 normal stomach and 414 primary tumours of stomach cancers, 165 normal testis and 154 primary tumours of testicular cancers.

Our results showed the deregulation of all four hernia susceptible genes *EFEMP1*, *WT1* and *EBF2* in colon, stomach and testicular cancers compared to their respective normal tissues except ADAMTS6, which is altered instomach and testicular cancers only (**Figures 1 to 4**). These results indicated the significance of hernia susceptible genes intervention in the development of cancers. These four hernia risk genes may have biological and pathological function in the hernia development which may have metabolic aetiology related to subtype of collagen and its maturation, elastin, and matrix metalloproteinase(Jorgenson et al. 2015). EFEMP1 gene encodes a member of fibulin family of extracellular matrix glycoprotein. Mice with EFEMP1 knockout developed inguinal hernias and showed the reduced elastic fibres in fascia(McLaughlin et al. 2007). Additionally, IPA analysis revealed the role of EFEMP1 on the connective tissue maintenance/homeostasis through its action on ECM enzyme matrix metalloproteinases that degrade collagen and elastin fibres(Jorgenson et al. 2015). EFEMP1 deregulation has been reported in solid tumours and showed as a weapon for 21st century cancer therapeutics through its tumour suppressor activity in extracellular matrix compartments (Zhou et al. 2016). We found significant down regulation of EFEMP1 in primary tumour of colorectal and stomach cancers (**Figures 1 and 2**) but increased in testicular cancers (**Figure 3**) suggest that EFEMP1 may be acting as tumour suppressor in colorectal and stomach cancers but not in testicular cancer. Since EFEMP1 association with connective tissue maintenance is known, the decreased expression in colorectal and stomach cancers suggest that these patients may be more sensitive to the development of hernia.

Wilm'stumor gene WT1 expression is upregulated in colorectal and stomach cancers but decreased in testicular cancers compared to their normal tissues (**Figures 1-3**). WT1 gene expression has been shown in be associated with the development of congenital diaphragmatic hernia(Dingemann et al. 2011; Carmona et al. 2016). The transcription factor early B-cell factor 2 (EBF2) expression was downregulated in colorectal and testicular cancers but upregulated in stomach cancers compared to their respective normal tissues (**Figure 1-3**). There is only one report indicating the role of EBF2 in hernia development (Jorgenson et al. 2015). EBF2 has been shown as an essential regulator of brown adipogenesis through tissue-specific chromatin remodelling complex(Shapira et al. 2017). ADAMTS6 was upregulated in stomach cancers and downregulated in testicular cancers, but there is not significant difference in colorectal cancers primary tissues compared to their respective normal tissues (**Figure 1-3**). Specific functions of a disintegrin and metalloprotease with thrombospondin motifs ADAMTS6 on hernia development are not known. Deregulation of ADAMTS6 is known in colorectal cancer(Xiao et al. 2015). It is advisable that the expression of hernia susceptible genes in cancers of colorectal, stomach and testis need to be considered while selecting the treatment option in these patients. Since hernia susceptible genes functions have been demonstrated in connective tissue maintenance and/or homeostasis, the expression of these genes may have significant effect on the treatment outcome and possible side effects when herniation occurs in these cancer patients. In conclusion, we have showed the differential expression of hernia susceptible genes in cancers of colorectal, stomach and testicular cancers. Our data suggest the association of hernia susceptible genes function in the development of hernia in cancer patients and these genes may be associated with the cancer therapeutic interventions. Further studies are required to understand the precise functions of hernia susceptible genes in cancers.

FIGURE LEGENDS

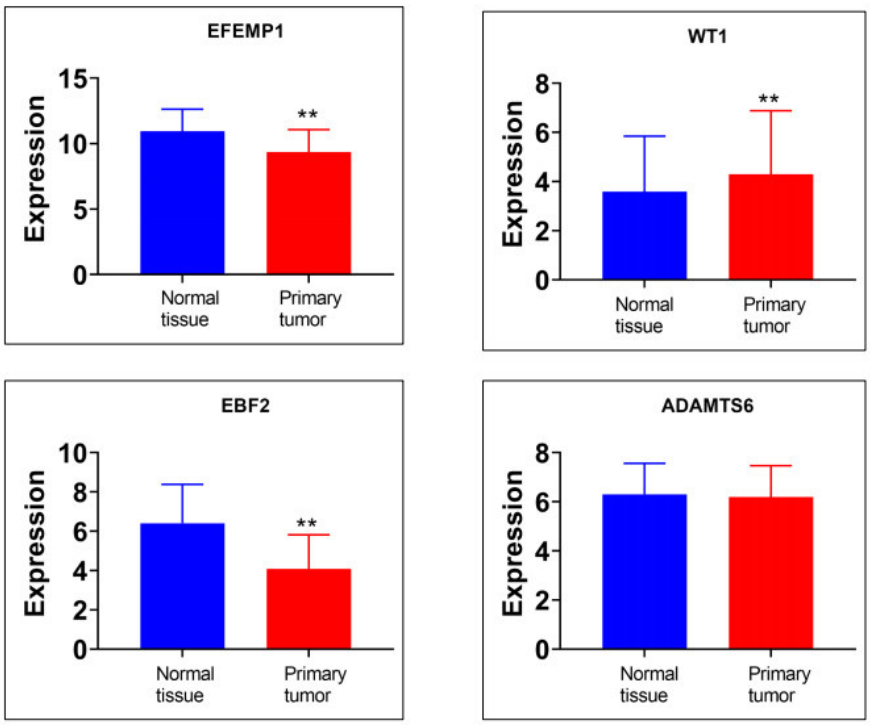

Figure 1.Differential expression of hernia susceptible genes in colorectal cancer compared to normal colon

Normalized mRNA expression of *EFEMP1*, *WT1*, *EBF2* and *ADAMTS6* was presented in normal colon tissues (n=308) and primary tumour tissues (n=288). Statistical significance of differential expression was determined by *t*-test by selecting Mann-Whitney test and comparison ranks. Statistical significance was showed as pvalues **p<0.0001.

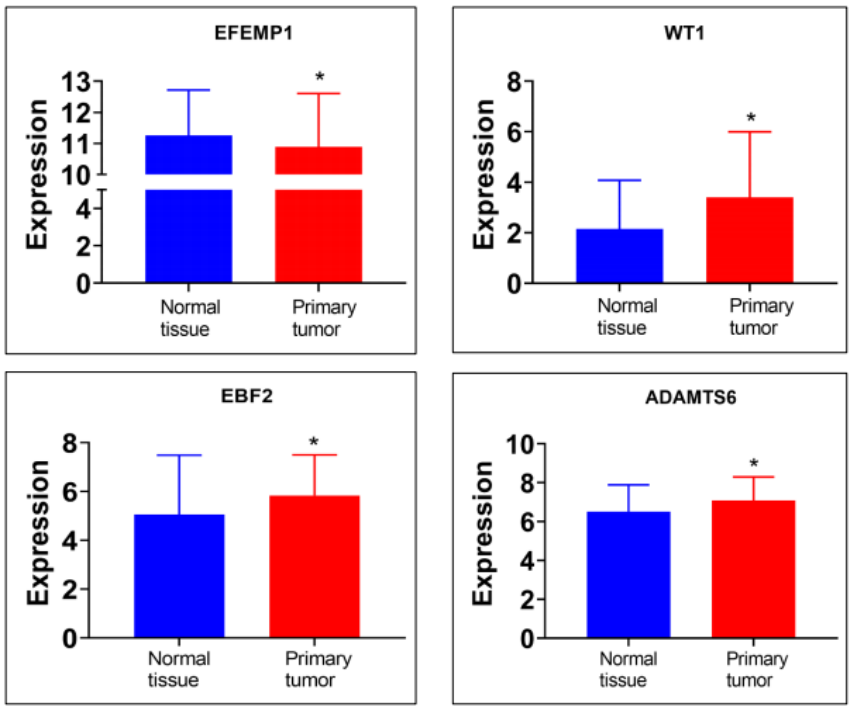

Figure 2. Hernia susceptible genes were differentially expressed in stomach cancer primary tumour tissues compared to normal stomach

NormalizedmRNA expression of *EFEMP1*, *WT1*, *EBF2* and *ADAMTS6* from 174 normal stomach and 414 stomach cancer primary tumour tissues were downloaded from TCGA database. Average expression data was presented with standard deviation. Statistical analysis was performed to determine the significance using *t*-test by selecting Mann-Whitney test and comparison ranksand p values were showedas *p<0.0.0001 significant.

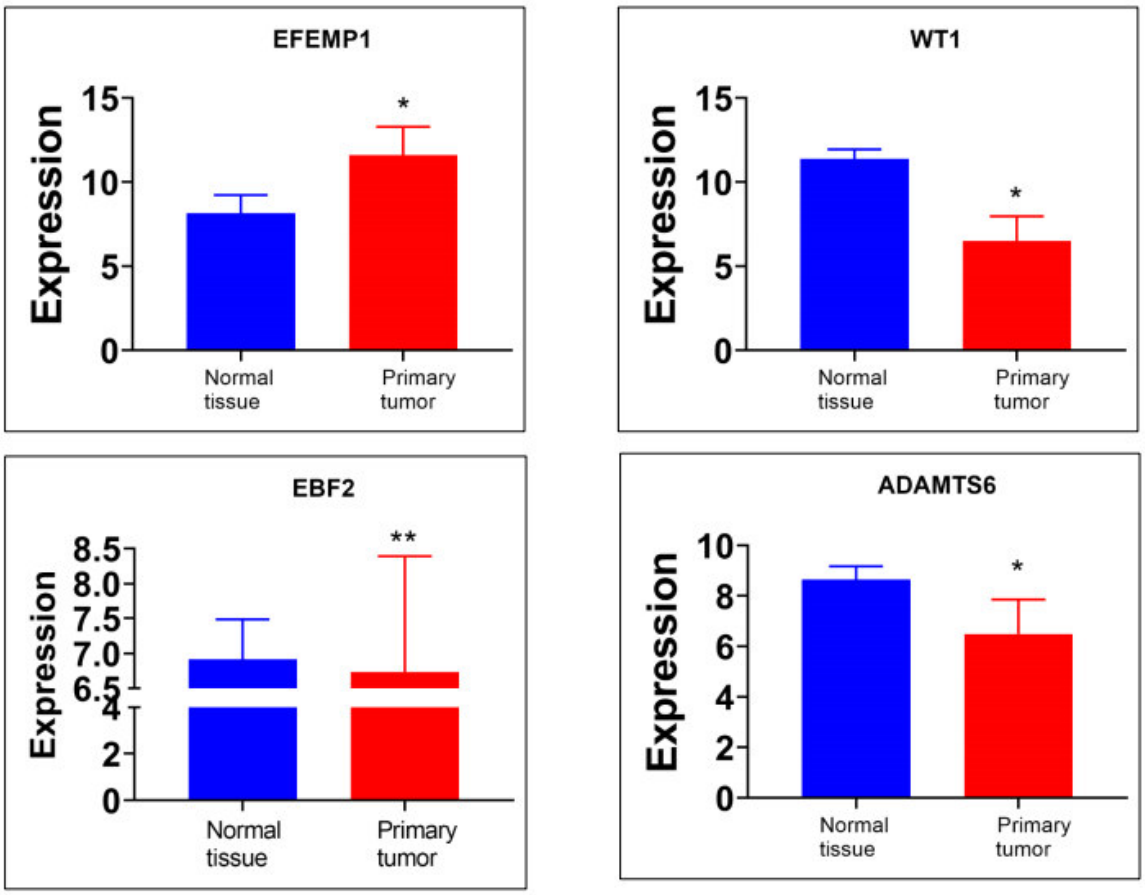

Figure 3. Expression of hernia susceptible genes were significantly altered in testicular cancer compared to normal testis

Normalized mRNA expression of *EFEMP1*, *WT1*, *EBF2* and *ADAMTS6* was obtained from the TCGA Target GTEx database using UCSC xenabrowser and showed the average expression from 165 normal testis tissues and 154 testicular primary cancer tumour tissues. Statistical analysis was performed using *t*-test by selecting Mann-Whitney test and comparison ranksto determine the significance of differential expression between normal and cancer and *p values <0.0001, **p<0.04 were considered as significant.

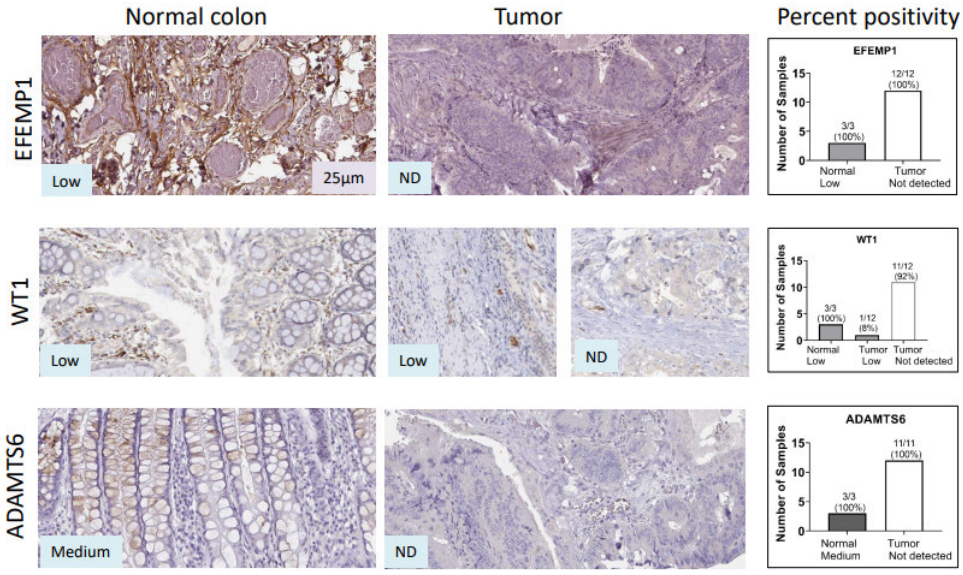

Figure 4. Immunohistochemical evaluation of hernia susceptible genes encoded proteins in normal colon tissues and colorectal cancer primary tumour tissues

The Human Protein Atlas database was utilized, downloaded the photomicrographs of IHC detected EFEMP1, WT1 and ADAMTS6 to determine the expression differences between normal (n =3) and tumour tissues (n=12). The magnification of photomicrographs was 25µm. Expression intensity was showed as not detected (ND), low, and medium. Percent positivity of each protein was showed in normal and tumour tissues (right panel). EBF2 expression data was not available in HPA database.

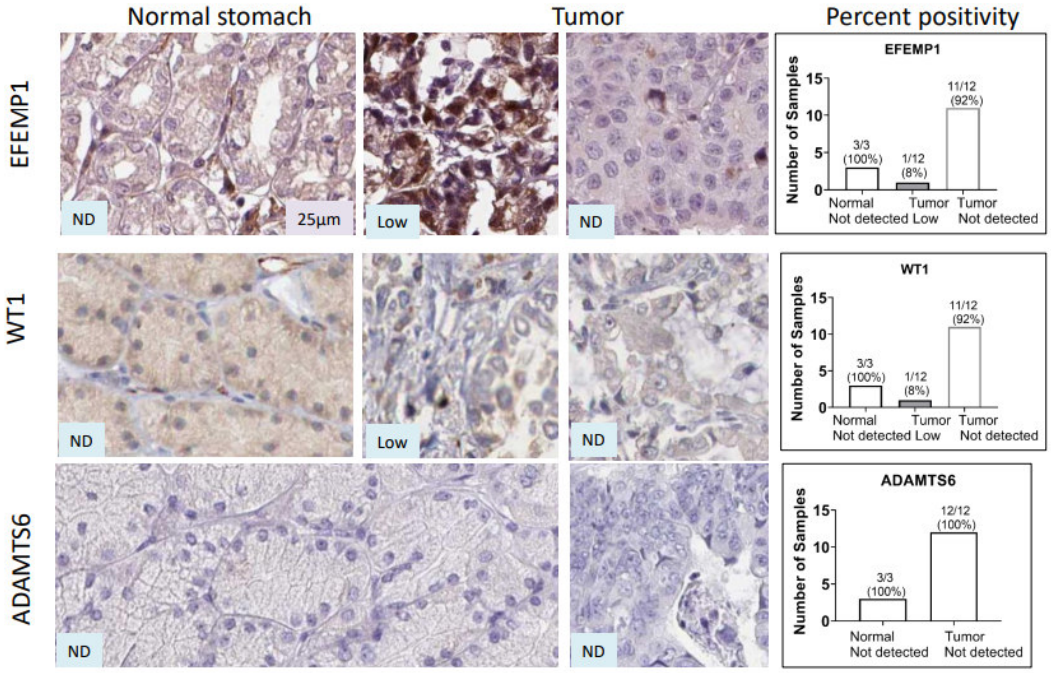

Figure 5.Detection of hernia susceptible proteins expression by Immunohistochemistry in normal stomach tissues and stomach cancer primary tumour tissues

IHC detected photomicrographs for EFEMP1, WT1 and ADAMTS6 from normal stomach (n=3) and primary tumour tissues (n=12) were downloaded at 25um magnification from The Human Protein Atlas database. Expression intensity was showed as not detected (ND) and low. Percent positivity of each protein was showed in normal and tumour tissues (right panel). EBF2 expression was not available in HPA database

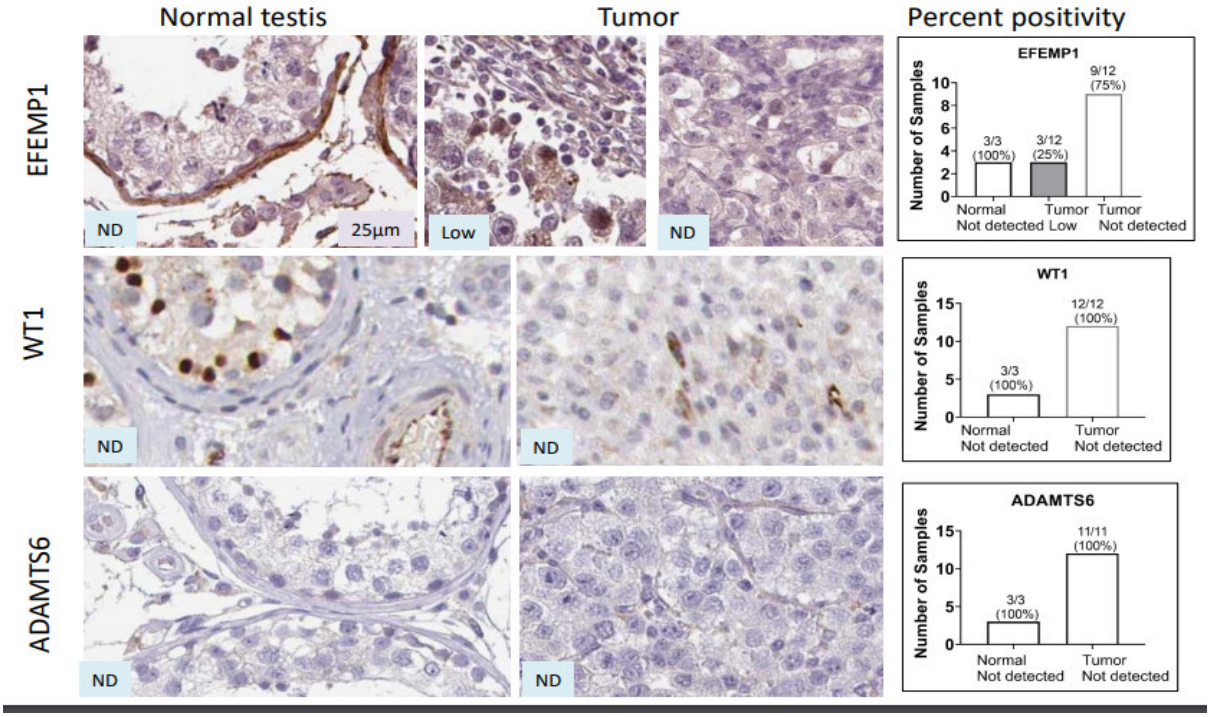

Figure 6. Hernia susceptible proteins expression in normal testis tissues and testicular cancer primary tumour tissues

Photomicrographs ofimmunohistochemically detected expression of hernia susceptible proteins EFEMP1, WT1 and ADAMTS6 were downloaded from The Human Protein Atlas database in normal testis (n =3) and primary tumour tissues (n=12). The magnification of photomicrographs was 25µm. Expression intensity was showed as not detected (ND) and low. Percent positivity of each protein was showed in normal and tumour tissues (right panel). EBF2 expression data was not available in HPA database.

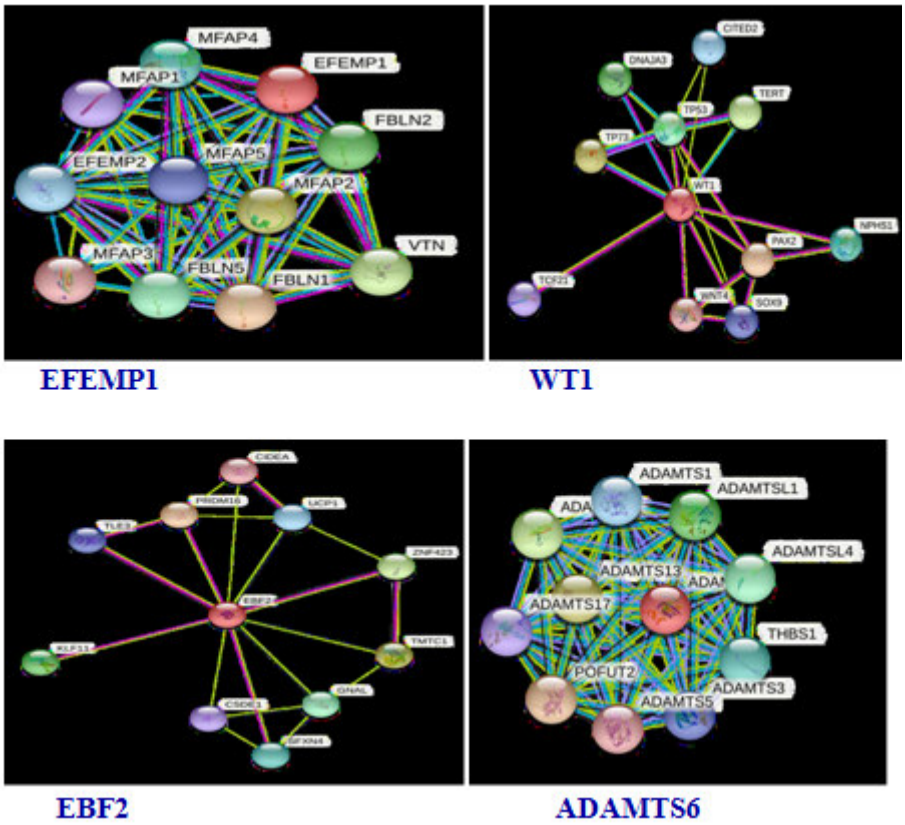

Figure 7. Protein-Protein interaction of hernia susceptible proteins

Edges represents protein-protein associations

Known interactions	Predicted interactions	Others
From curated database Experimentally determined	Gene neighbourhood Gene fusions Gene occurance	Textmining Co-expression Protein homology

Human hernia susceptible proteins EFEMP1, WT1, EBF2, and ADAMTS6 were quarried for the interaction with other proteins using the STRING database. The interactions were showed as nodes represented proteins produced by single protein-coding gene and edge represented the protein-protein interactions. Colour nodes represented the first shell of interactions, Different colour edges represented different type of protein-protein interaction including known interactions, predicted interactions and others

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How to cite this article: Jyothsna Bollu and Srikanth Pallothu. Alteration Of Hernia Susceptible Genes In Colorectal, Stomach And Testicular Cancers. Int J of Allied Med Sci and Clin Res 2021; 9(3): 571-582.

Source of Support: Nil. **Conflict of Interest:** None declared.