Expression Of Tenascin In Infertility And Possible Link To Ovarian And Breast Cancer

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ABSTRACT

BACKGROUND

AIM: To Investigate the Expression of Tenascin in infertility and possible link to Ovarian and Breast Cancer

MATERIALS AND METHODS: Twenty-four women with infertility as an issue have undergone laparoscopic procedures infertile women at the Medical Health and Research Institute between February 2023 and March 2024 were recruited. The analysis comprised blood samples from Twelve women diagnosed with endometriosis (EM) and twelve without endometriosis who underwent surgery, with both groups undergoing biopsies.

Blood samples were collected on the same day of surgery, during fasting, and were sent to the laboratory for subsequent analysis of protein expression in distinct groups, namely the endometriosis group and the control group.

RESULTS: In this study, we found four proteins that were expressed highly in severe forms of Endometriosis are MLLT4, ZC3H13, Laminin Subunit-1, and Tenascin. We found that there is a seven-fold increase in Tenascin levels which may be associated in infertility

CONCLUSION: The up-regulation of tenascin could play various roles in the pathogenesis of endometriosis. One such role could involve contributing to the abnormal attachment or proliferation of endometriotic cells. Tenascin may play a role in the implantation process and could be associated with the infertility mechanisms implicated in endometriotic disease.

Keywords: Endometriosis, Tenascin, Upregulation, Infertility

Introduction

Endometriosis is characterized by the presence of endometrial stromal and glandular cells outside the uterine cavity, primarily located within the pelvic region [1]. It is a chronic inflammatory disease that depends on estrogen and affects approximately 10-15% of women of reproductive age. The precise etiology and pathophysiology of the disease remain unclear. However, the widely accepted theory implicates retrograde menstruation, leading to the implantation of endometrial cells on the peritoneal surface.[2]

Recently several studies have reported molecular network and signaling pathways play a crucial role in unexplained infertility in severe forms of endometriosis. The preservation of the structural and functional integrity of maternal uterine tissue is essential for successful implantation and conception. The molecular environment within the uterus influences pregnancy establishment and development, with key components such as extracellular matrix (ECM) components, adhesion molecules, cytokines, and growth factors contributing to structural support and cellular processes which contributes to disease progression. Hence, a comprehensive understanding of the composition and expression patterns of protein expression, in comparison to that of corresponding healthy tissues,

holds significant clinical significance. Targeting disease specific ECM molecules in conjunction with traditional treatments could offer targeted treatment to patients [3]. Tenascin is an extracellular matrix glycoprotein that plays roles in tissue remodeling and cell adhesion. Upregulation or increased expression of Tenascin has been associated with various pathological conditions, such as endometriosis, pelvic inflammatory disease, and uterine fibroids which impact fertility [4]

The development of endometriosis likely entails cell adhesion and local invasion into the underlying tissue. Since the extracellular matrix (ECM) plays a pivotal role in regulating these cellular events, it is conceivable that endometriosis could be linked to dysregulated cell-ECM interactions. Endometriosis is characterized by the presence of endometrial stromal and glandular cells outside the uterine cavity, primarily in the pelvis. Despite extensive research, the exact etiology and pathophysiology of the disease remain elusive.

The prevailing theory implicates retrograde menstruation and the subsequent implantation of endometrial cells on the peritoneal surface. Endometriotic cells possess the ability to adhere to the peritoneal mesothelium, breach the peritoneal lining, and disrupt the extracellular matrix (ECM), thereby infiltrating surrounding tissues. Given the pivotal role of the ECM in regulating these cellular processes, ECM invasion may be a crucial aspect of endometriotic development.

Tenascin, an extracellular matrix (ECM) glycoprotein, is widely distributed throughout the body and contributes to various cellular processes including differentiation, proliferation, and migration. Its expression is associated with embryogenesis, indicating its potential involvement in regulating epithelial-mesenchymal interactions crucial for tissue development. In the human endometrium, Tenascin expression undergoes physiological changes throughout the menstrual cycle. During the proliferative phase, Tenascin is predominantly deposited in the stroma surrounding the endometrial glands. However, its specific role in the endometrium and its implications in conditions like endometriosis remain poorly understood

The Tenascin comprise a family of modular extracellular matrix (ECM) glycoproteins which encompass four members. The extracellular matrix (ECM) surrounding cancerous cells, the founding member of the Tenascin family are TN-C, where the "C" denotes "cytotactin," one of its initial names. Subsequently, tenascin-R (TN-R), tenascin-X (TN-X), and TN-W Were tenascin-C and tenascin-W emerged as the most viable options for potential exploitation and clinical application.(**as depicted in Figure 1**)[5,6]. This is attributed to their elevated expression levels within diverse tumor stromal environments, juxtaposed with their comparably sparse presence in healthy tissues.

Tenascin are categorized as regulators of cell adhesion, migration, and growth. Within tenascin, Type III modules, as well as those found in fibronectin, interact with VEGF, promoting cell proliferation in an integrin-dependent fashion. Tenascin plays a crucial role in regulating both the endometrial menstrual cycle and tissue breakdown during menstruation. Additionally, it facilitates the attachment and invasion of endometrial cells to the extracellular matrix [7].

TN-C and TN-W are both observed in association with endothelial cells in numerous disease types, indicating the potential involvement of both tenascins in facilitating tumorangiogenesis.

Tenascin-C plays a regulatory role in Tumor angiogenesis, and immune suppression, promotes plasticity, invasion, and metastasis, shapes tissue and tumor immunity. Tenascin-C (TNC) serves as a pivotal factor in tissue remodeling, and its dysregulated overexpression is closely associated with numerous diseases, such as heart failure, thrombosis, atherosclerosis, and cancer [8]. Elevated TNC expression has been observed in the majority of solid cancers, spanning various organs including the brain, breast, uterus, prostate, pancreas, colon, stomach, mouth, larynx, lung, liver, kidney, bladder, skin, bone, soft tissues, lymphomas, and ovaries. [9].

TN-C exhibits versatile interactions with various molecules, engaging distinct cell surface receptors such as toll-like receptor 4 (TLR4) and integrins, along with matrix components like fibronectin (FN), collagens, and lecticans, as well as soluble factors including growth factors and chemokines. These interactions modulate diverse cell type-specific and context- dependent responses [10] While TNC expression is typically tightly regulated, in cancer, it is markedly upregulated around blood vessels, within immune suppressive matrix niches, at the invasive front, and in metastatic sites [11]. The Upregulation of tenascin expression in both infertility and cancer can be ascribed to the intricate interactions within the microenvironment of either the tumor or reproductive tissue. These interactions commonly entail the activation of multiple cellular signaling pathways and the involvement of various molecular factors.

In infertility, the upregulation of tenascin may contribute to changes in the microenvironment of the reproductive organs [12].These changes could potentially lead to impaired embryo implantation or compromised sperm motility. Additionally, tenascin may be involved in the development of conditions like endometriosis, which is known to be associated with infertility.[7]

Targeting tenascin or its signaling pathways could potentially be a therapeutic approach for infertility associated with conditions like endometriosis. By inhibiting tenascin expression

or its downstream effects, it may be possible to restore normal reproductive function and improve fertility outcomes

Tenascin, a high molecular weight ECM protein, plays a critical role in tissue regeneration, hyperplasia, and neoplastic processes. It is implicated in angiogenesis through its influence on the migration of vascular endothelial cells. The expression of tenascin in the endometrium undergoes fluctuations under the influence of ovarian steroids during the menstrual cycle. Although the exact function of endometrial tenascin remains unclear, it is hypothesized to play a role in endometrial regeneration and the implantation of endometriotic lesions through cellular adhesion In this study, our objective is to explore the expression patterns of tenascin in both eutopic and ectopic endometrial tissues and to check the inflammation, invasion and migration and tissue proteomics was done to understand it.

2. Materials and Methodology

Twenty-Four women with infertility as an issue have undergone laparoscopic procedures infertile women at the Medical Health and Research Institute between February 2023 and March 2024 were recruited. Informed consent was obtained from all participants following authorization of the protocol by the Institutional Review Board. Eligible female subjects were under 40 years old with regular menstrual cycles (27–35 days), typical basal body temperature patterns, normal serum prolactin levels, and male partners with ≥5 million motile sperms, as per our study criteria.

The investigation into the cause of infertility followed a standardized protocol, including hormonal and biochemical profiling, testing for sexually transmitted diseases, imaging examinations, and assessment of genetic and/or immunological factors. Semen analysis, hysterosalpingography, hysteroscopy, and laparoscopy were performed, with laparoscopy conducted for all women up to 36 years old and selectively for those over 36 based on symptoms or abnormal imaging findings. Infertility was classified as unexplained if no abnormalities were detected after these assessments. Women failing to conceive after at least six natural or induced cycles post-laparoscopy were deemed infertile.

The control group comprised fertile women with a history of parity and no uterine abnormalities, all of whom abstained from steroidal medications for the prior six months. Exclusions were made for women with hyperprolactinemia, polycystic ovarian syndrome (PCOS), or moderate-severe endometriosis. Age, basal serum follicle-stimulating hormone (FSH) levels, and body mass index (BMI) did not significantly differ between groups.

Clinical data and tissue samples were collected following a detailed explanation of the study's objectives, with participants consenting via forms approved by the Research Ethics Committee of the Medical Health and Research Institute (MHRI).

Sample Preparation

During the mid-secretory phase of the menstrual cycle (LH +7), biopsies of endometrial tissue were aseptically obtained and stored at -80°C for proteome analysis. Serum samples underwent depletion using the ProteoPrep Blue Albumin and IgG Depletion Kit (SIGMA- ALDRICH), and their protein content was determined via the Bradford test. Frozen endometrial tissue samples (40mg) were homogenized with 400µl of urea lysis solution using a handheld homogenizer (POLYTRON® System PT 1200E), followed by vortexing and sonication. After centrifugation, the clear supernatant was stored at -80°C.

For the first dimension of separation, pH 3-10 13cm IPG strips from GE Healthcare were used, and proteins were focused on an IPGPhor III apparatus. Each IPG strip underwent isoelectric focusing (IEF), followed by equilibration and second dimension PAGE (12.5%) using the SE600 system. Protein spots were stained with colloidal Coomassie blue G-250 and analyzed using PDQuest 8.01. Mass spectrometric analysis was performed on target protein spots excised from the gel after trypsin digestion.

Protein identification was conducted using Matrix-assisted Laser Desorption Ionization (MALDI-TOF/TOF) Mass Spectrometry and LTQ-Orbitrap XL. Proteins with a MASCOT score greater than 64 and more than four peptide matches were considered significant. Proteins exhibiting a minimum 1.5-fold change between groups and a P-value of 0.05 underwent further bioinformatic analysis.

Statistical analysis was performed using Student's t-tests in SPSS for IBM Statistics version

20. Only proteins showing consistent and significant changes were included for subsequent bioinformatics analysis. Pathway and network analyses were conducted using right-tailed Fisher's exact tests, with a significance level of $P<0.05$ utilized for all analyses.

2.1. Sample Collection

Tissue samples were collected from endometriosis patients and control patients with eutopic endometrium from January 2023 to December 2023. Eutopic and ectopic endometrial samples were procured from 45 women aged 18–40 years, with a body mass index ranging from 18.5 to 24.9 kg/m², undergoing laparoscopy for benign indications. These women had regular menstrual cycles lasting 26–33 days and had not received hormonal medication in the preceding 3 months. The participants were divided into two groups: Group I comprised women without pelvic pathology confirmed by ultrasonography and laparoscopy (n = 12), while Group II included women diagnosed with endometriosis (n = 12). Samples of endometriotic implants and endometriomas were obtained during laparoscopy,

with disease confirmation via histological examination. Eutopic Endometrium was obtained from endometrial biopsies performed during surgery. Histological examination of sections from these samples was conducted, evaluating for proliferative or secretory endometrial morphological features based on the criteria established by Noyes et al. Immunohistochemical and enzyme immunoassay analyses were performed using 24 samples of endometrium from 12 women with a normal pelvis and 12 women with endometriosis. Semiquantitative estimation of tenascin staining was conducted to evaluate staining intensity and distribution. Peripheral venous blood samples (5mL) were collected for enzyme immunoassay. Written informed consent was obtained from each patient using consent forms.

DE and Image Analysis

In the first step, we utilized pH 3-10 13cm IPG strips sourced from GE Healthcare, Uppsala, Sweden, and conducted active/passive rehydration. Proteins underwent focusing on an IPGPhor III apparatus (GE Healthcare, Uppsala, Sweden) under the following IEF conditions: a 100 V gradient for 1 hour, a 300 V gradient for 2 hours, a 1000V gradient for 1 hour, a 5000V gradient for 5 hours, and a 5000 V step, followed by a 5000 V step maintained for 7 hours at a constant temperature of 20 °C. Each IPG strip was subjected to isoelectric focusing (IEF), followed by equilibration with 2% DTT and subsequent incubation with a different buffer containing 2.5% iodoacetamide in lieu of DTT. For the second dimension PAGE (12.5%), we employed the SE600 system (GE Healthcare, Uppsala, Sweden) at 1W/gel for 1 hour and 13W/gel for 3 hours. Protein spots were stained using colloidal Coomassie blue G- 250 and then scanned utilizing a high-precision scanner (ScanMaker 9700XL, Microtek). Subsequently, gel images underwent analysis utilizing the gel image analysis tool PDQuest

8.01 (Bio-Rad) [19]. Mass spectrometric analysis was conducted on the targeted protein spots excised from the gel post-trypsin digestion. Gel fragments were rinsed with Milli-Q water and treated with a decolorizing solution comprising 50% acetonitrile and 25% ammonium bicarbonate. Following dehydration in 100% acetonitrile (ACN) for 10 minutes, the decolorized gel particles were vacuum dried for 30 minutes.

Protein Identification and Data Analysis

Following excision from the gel, protein spots underwent decolorization, digestion, and extraction as outlined by [13] . Peptide samples were subjected to analysis using Matrix-assisted Laser Desorption Ionization (MALDI-TOF/TOF) Mass Spectrometry and LTQ-Orbitrap XL (Bruker Daltonics, Bremen, Germany). Protein identification was conducted utilizing Bio Tools 3.0 software on MASCOT (V2.1, Matrix Science, UK), relying on peptide mass fingerprint data. Proteins with a MASCOT score exceeding 64 and comprising more than four peptide matches were deemed significant ($P<0.05$). Proteins demonstrating a minimum 1.5-fold change between groups and a P-value of 0.05 were subsequently subjected to additional bioinformatic analysis.

Statistical Analysis

Clinical and experimental data underwent analysis utilizing Student's t-tests performed in SPSS for IBM Statistics version 20 (IBM Corp., Armonk, NY, USA). Only proteins demonstrating consistent and significant changes (either increased or decreased) were selected for subsequent bioinformatics analysis. P-values were computed using right-tailed Fisher's exact tests, as appropriate, to identify statistically significant pathways and networks associated with the identified proteins. A significance level of $P<0.05$ was applied to all analyses [14]

Results: The study analyzed a total of twenty-four patient samples, comprising twelve samples of endometrial tissue and blood from patients diagnosed with endometriosis (EM) and six samples from patients without endometriosis obtained during surgery. The baseline characteristics of the patients are detailed in Table 1. Notably, the age of patients in the control group exceeded that of the endometriosis group, and both groups had a higher proportion of Asian patients compared to other ethnicities. Additional clinical characteristics such as parity, gravidity, rARSM stage of endometriosis patients, and clinical diagnosis in the control group are provided in Table 1. Of particular concern is the prolonged duration of infertility among individuals with endometriosis compared to the controls

In this study, we found four proteins that were expressed highly in severe forms of Endometriosis are MLLT4, ZC3H13, Laminin Subunit-1, and Tenascin. Their function and related roles are described in **Table No 2 and Figure 2**

There is a sevenfold increase in Tenascin protein levels associated with infertility could indicate several potential scenarios like Inflammation and tissue remodeling, Endometriosis, Ovarian dysfunction, Fallopian tube dysfunction, and Implantation failure. It's important to note that while elevated levels of Tenascin may be associated with infertility, correlation does not necessarily imply causation. Using the Pharos version 3.18, we characterized the upregulated common proteins found in blood and tissue samples of endometriosis patients and the control group based on their biological processes, cellular components, and protein classes where the Classification of Targets in the Hierarchical Families according to Drug Target Ontology as shown in **Figure 3** , GO Process: Cellular processes involving targets in the list, as defined by UniProt in **Figure 4.** the role of Overlap Associated Diseases in relation to Tenascin as depicted in **Figure 5**

Data Analyzed following protein extraction and Matrix-assisted Laser Desorption Ionization (MALDI-TOF/TOF) Mass Spectrometry and LTQ-Orbitrap XL

(Bruker Daltonics, Bremen, Germany) showed. Protein identification was performed using the Bio Tools 3.0 software on MASCOT (V2.1, Matrix Science, UK) based on the peptide mass fingerprint data. Proteins with a MASCOT score greater than and more than four peptide matches were considered significant

Discussion

In our Proteomics data analyzed we have shown that MLLT4, ZC3H13, laminin subunits, and Tenascin and we found that there was seven fold increase in expression levels in each menstrual cycle, the human endometrium undergoes significant changes, and extracellular matrix (ECM) proteins play a pivotal role in this process by regulating cell differentiation, adhesion, proliferation, and migration. The precise causes of infertility associated with endometriosis remain unclear despite its recognized significance as a contributing factor in women of reproductive age [15].Few Research Studies have suggested that tenascin may play a role in regulating ovarian function and folliculogenesis, which are essential processes for follicle development in the ovary[16]. Tenascin plays a dual role in the regulation of the endometrial menstrual cycle. It contributes to tissue breakdown during menstruation and is also involved in facilitating the attachment and invasion of endometrial cells to the extracellular matrix (ECM)[17].

Abnormalities in folliculogenesis have been associated with infertility. Studies have indeed suggested a link between endometriosis, elevated levels of Tenascin-C, and infertility ie: Tissue Remodeling and Inflammation, Adhesion Formation, Implantation Failure, Inflammatory Microenvironment, and Angiogenesis [18]. Furthermore, tenascin expression has been identified in the endometrium, the inner lining of the uterus, where it may contribute to the regulation of implantation and early pregnancy. Any disruption in tenascin expression within the endometrium could potentially impact fertility. [19] The modulation of tenascin, an extracellular matrix protein, by estrogen (E2) in endometriotic stromal cells may be one of the contributing factors in the development of endometriosis. [20]The upregulation of tenascin may play multiple

roles in the pathogenesis of endometriosis. One such role is its potential contribution to the aberrant attachment or proliferation of endometriotic cells. Understanding the intricate interplay between extracellular matrix (ECM) proteins and estrogen is essential for comprehending normal endometrial physiology and for devising targeted treatments for endometriosis.[21] The disease bears resemblance to malignant conditions due to its involvement in cell migration, invasion, and proliferation.

Tenascin-X (TNX) is a large extracellular matrix protein discovered because its TNXB gene overlaps the CYP21A2 gene encoding steroid 21-hydroxylase (P450c21). Sequencing of the structure of a tenascin: N-terminal EGF-like repeats, multiple fibronectin-III repeats, and a C-terminal fibrinogen-like domain; we named this “Tenascin-X” (TNX). To study TNX function, we postulated a “contiguous gene syndrome”—a single mutation affecting both CYP21A2 and TNXB, causing CAH plus another disorder that might suggest the role of TNX.

Further manual sequencing demonstrated that the structure contained the five domains expected of a Tenascin. First, the N-terminus comprised a 22AA signal peptide that directs the protein to the secretory pathway, used by extracellular matrix proteins. Second, is a hydrophobic domain containing three heptad repeats that encode the Tenascin “headpiece”, which permits the polymerization of Tenascin monomers into multi-armed “brachion” structures.

MLLT4, ZC3H13, laminin subunits, and Tenascin, may indeed have interactions with mucins and play crucial roles in cell signaling, immune responses, and cell adhesion.

The findings of the study suggest that there is a seven-fold increase in Tenascin protein could potentially be associated with infertility, and they provide several potential scenarios or conditions where this association might be observed in comparison to **MLLT4, ZC3H13, laminin subunits**. There could be other underlying factors or mechanisms at play that contribute to both the elevated Tenascin levels and the infertility.

Tenascin levels could be elevated because of underlying pathological processes such as inflammation or tissue damage, which in turn may affect fertility.

Conclusion

The up-regulation of tenascin may have some functions on the pathogenesis of endometriosis. One such role may be able to contribute to the abnormal attachment or the proliferation of endometriotic cells. The complex relationship between ECM proteins and estrogen is important for understanding normal endometrial function and for the design of future targeted therapeutic approaches for endometriosis and estrogen modulation of tenascin expression in endometriotic stromal cells: Could this be one of the mechanisms contributing to endometriosis development**.** Finally, the relation of Tenascin expression in infertility and the possible connection to ovarian and breast cancer indicates the function of the protein in diverse pathological events.

Table 1 shows the Clinical Characteristics of Patients with Endometriosis and Controlpatients

Characteristics of the Patient with Endometriosis			
Base line parameters	**Endometriosis group**	**Control Group**	**P value**
Number of women	12	12	NA
Age (years mean ± S.D)	34±0.3	35.4 ±0.8	0.007a
Body mass index (mean ± S.D)	23.5 ±0.4	24.0 ±0.3	0.03a
Height(cm)	169±0.7	168±1	0.07a
Weight(kg)	65.6±8.0	64±7.0	0.72a
Ethnicity, n (%)			
Asian	8(66%)	9(75%)	0.55b
African American	3(25%)	2(16%)	>0.99b
Unknown	1(8%)	---	---
Parity, n (%)			
0	9(75%)	7(58%)	0.55b
≥1	3(25%)	4(33%)	>0.99b
Unknown	--	1(8%)	---
Gravidity, n (%)			
0	4(33%)	2(16%)	0.50b
≥1	8(66%)	10(83%)	0.50b
Unknown	--	--	--
Duration of infertility in years	12 ±2.5	11 ±3.1	0.22 a
rARSM Stage, n (%)			
Stage I(minimal)	1(8%)	NA	NA
Stage II(mild)	2(16%)	NA	NA
Stage III(Moderate)	3(25%)	NA	NA
Stage IV(Severe)	6(50%)	NA	NA

Table 2: Protein expression levels assessed in the unexplained infertile and fertile proteome found in Tissue samples as identified by MALDI-TOF/TOF or LTQ-Orbitrap XL mass. Only the most expressed proteins are reported.

Accession Number	Protein Description	Gene Symbol	Subcellular localization	Protein Score	Fold Change**	Ratio
Q96C95	MLLT4protein (Fragment) OS=Homosapiens OX=9606 GN=MLLT4 PE=2 SV=1	MLLT4		46.29	2.230831201	1.173510867
A0PJJ2	ZC3H13protein(Fragment) OS=Homosapiens OX=9606 GN=ZC3H13 PE=2SV=1	ZC3H13	Nucleus	165.38	2.024313448	2.033991215
A0A7I2V4J9	Laminin subunit beta-1 OS=Homo sapiens OX=9606 GN=LAMB1 PE=1 SV=1	Laminin subunit beta1	Extracellular membrane, extracellular matrix, basement membrane	8.54	2.428268104	2.691234498
A0A087WWA5	Tenascin-X OS=Homosapiens OX=9606 GN=TNXB PE=1 SV=1	Tenascin	Extracellular Matrix protein	26.79	7.271182951	154.4700091

Figure 1: Illustrates Organization of Tenascin

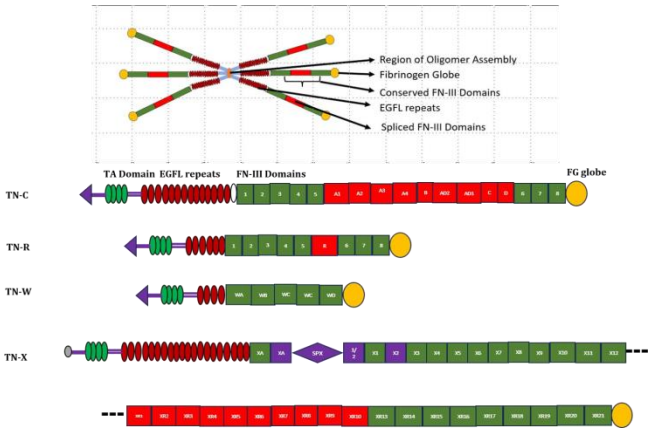


Figure 2: A graphical representation depicting the proteins that are significantly overexpressed, exerted on log fold change in severe form of endometriosis

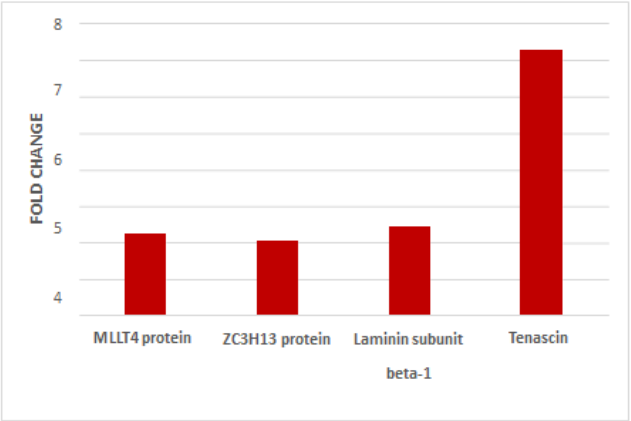


Figure 3: the Classification of Targets in the Hierarchical Families according to Drug Target Ontology

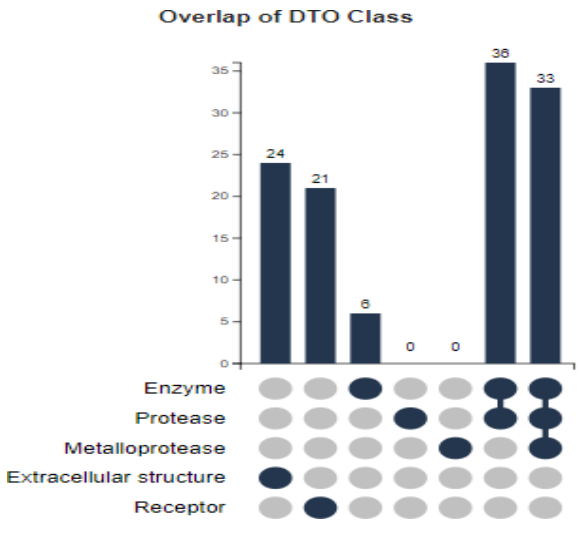


Figure 4: GO Process Cellular processes involving targets in the list, as defined by **UniProt**.

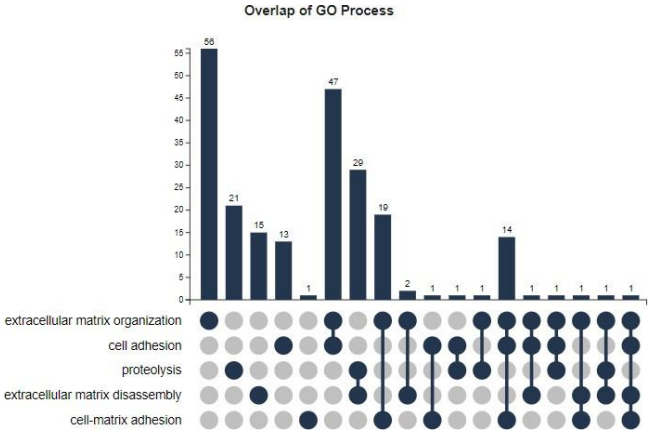
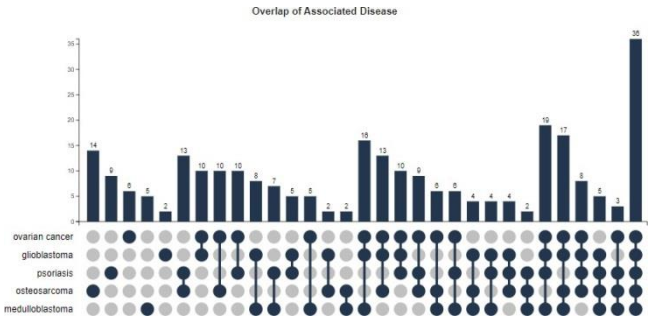


Figure 5 depicts the role of Overlap Associated Diseases in relation to Tenascin



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Competing interests: The authors declare no conflict of interest.

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