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Structure-Based Design of TFF3-PAR2 Inhibitor Peptides as A Promising New Therapeutic Approach for Endometriosis Patients

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ABSTRACT

Endometriosis features the presence of stroma and endometrial cells outside the uterine cavity, with a higher frequency within the pelvic compartment but may spread to other tissues and organs. Endometriosis may be detected in the ovaries, extensive adhesions obliterating the anterior or posterior cul-de-sacs, the broad ligament and uterosacral ligaments. Although endometriosis can be superficial, invading only the peritoneum, in some cases the disease can be invasive. This disease is driven by estrogen, affecting mainly reproductive age women between 25-35 years and decrease in women undergoing menopause due to the fact that the appearance of the lesions is dependent on hormones produced by the ovary. The treatment of this disease traditionally employs the use of hormones to alter its concentration in the patient causing a hypestrogenic state, being common the appearance of side effects. The endometriomas can be removed by laser or electrocautery when surgical methods do not work and the disease has progressed. Endometriosis is a multifactorial disease and its treatment most often involves the use of invasive methods, some strategies have been developed to improve the diagnosis and treatment of the disease. Here, we identified hot spot residues on the interface of interaction between TFF3 and PAR-2, both proteins related to inflammation and immune system. Moreover, TFF3 is known to be overexpressed in endometriosis and other diseases such as cancer. We designed structure-base peptides that intend to reduce the activity of TFF3 when this protein is overexpressed. We believe that inhibitor peptides could be a promising new approach of treatment for endometriosis in the future.

KEYWORDS

Endometriosis; TFF3; PAR-2; Polymorphism; Inhibitor Peptides.

INTRODUCTION

Endometriosis features the presence of stroma and endometrial cells outside the uterine cavity, with a higher frequency within the pelvic compartment but may spread to other tissues and organs [1]. The endometriosis is a chronic, inflammatory condition, associated with chronic pelvic pain, infertility, dysmenorrhea and dyspareunia. The pathogenesis of endometrioses is not fully understood, evidence indicates that the disease is possibly associated with changes in the immunity, retrograde menstruation, metastatic spread and coelomic metaplasia. In addition genetic and environmental factors are also associated with onset and development of the disease [2].

Endometriosis may be detected in the ovaries, extensive adhesions obliterating the anterior or posterior cul-de-sacs, the

broad ligament and uterosacral ligaments. Although endometriosis can be superficial, invading only the peritoneum, in some cases the disease can be invasive. Patients with invasive endometriosis often show endometriomas within the ovaries and deep lesions that invade into bladder and bowel. It is rarely detected in the brain, liver and lung. This clinical picture is associated with more advanced stages of the disease [3].

This disease is driven by estrogen, affecting mainly reproductive age women between 25-35 years and decrease in women undergoing menopause due to the fact that the appearance of the lesions is dependent on hormones produced by the ovary [3]. Interestingly, several studies have demonstrated the emergence of invasive endometriosis in adolescents [4].

Formerly, the gold-standard of the diagnosis was by surgical identification usually by laparoscopy [3]. However, in many cases women of reproductive age do not undergo surgical procedure and the use of drugs that delay the development of the disease and reduce pelvic pain are indicated [5]. Non-invasive methods such as imaging tests have been recently used for the diagnosis of endometriosis [6]. The gynecologic bimanual examination and the intravaginal ultrasound are able to detect rectovaginal plaques, deep nodules and ovarian endometriomas with efficiency [7].

The treatment of this disease traditionally employs the use of hormones to alter its concentration in the patient causing a hypestrogenic state, being common the appearance of side effects. The endometriomas can be removed by laser or electrocautery when surgical methods do not work and the disease has progressed. Other methods, such as hysterectomy or bilateral oophorectomy, are performed. The latter method is not usually employed in women in the reproductive age, since hormones are important for processes such as maintaining vaginal mucosa, bone density and sexual drive [3].

Therapy employs non-steroidal anti-inflammatory drugs, being the first line of treatment of symptoms. They inhibit the production of prostaglandin, repressing the COX enzyme involved in arachidonic pathway leading to a decrease in pelvic-pain [8], another form of treatment include progesterone therapy, levonorgestrel, contraceptive injection with depo-provera, gonadotropin-releasing hormone agonists which acts by inducing an increase in the production of the luteinising hormone (LH) and follicle-stimulating hormone (FSH) and danazol [3].

Several works has showed that gene polymorphisms might associated with the development of endometrioses. *CYP1A1* polymorphisms, for example, associated with increased risk of endometrioses specifically in Asia [9]. Meta-analyzes revealed that the *rs7521902* SNP (single nucleotide polymorphism) of the gene *WNT4* is associated with more severe cases of the disease [10] this gene plays a decisive role during the embryonic development of the female genital tract [11]. Therefore, the variant of this gene may favor the development of endometriosis by abnormal growth of female genital tissue [10].

Mutation of genes are also associated with infertility in the endometriosis, analysis of the AMH gene revealed that a SNP within its sequence is associated with infertility in endometriosis. AMH codes for a glycoprotein that inhibits the development of the female reproductive tract, or the Müller ducts in the male embryo, blocking the development of the fallopian tubes, uterus, and upper vagina, [12]. Other polymorphic genes are also associated with an increased risk of developing endometriosis such as such as IL-16 [13], interleukin 12B [14], Arylamine N-acetyltransferase 2 (*NAT2*) [15], *CYP1A1* [16,17], *p53* [17-19], *eNOS* [18].

Although endometriosis is a multifactorial disease and its treatment most often involves the use of invasive methods, some strategies have been developed to improve the diagnosis and treatment of the disease; in this sense some studies have employed the use of peptides as drug for the treatment and molecular markers for the diagnosis [16-19] of the disease. Linzh and colleagues, 2018 employing the phage display technology described a peptide with high affinity to bind to ectopic endometrium cells, possibly the use of this peptide may favor treatment and prognosis of endometriosis [20].

MATERIALS AND METHODS

The protein 3-D structures used in the analysis are available in the PDB (protein databank; https://www.rcsb.org/). The KBDOCK server was used to identify protein domains and interaction between protein domains [21]. The protein-protein docking was performed by the Gramm-X [22, 23] server. Briefly, the protein-protein docking is based on thermodynamics in order to find the best conformational structure at the minimum free energy, which would reflect the most probable PPI (protein-protein interaction) binding complex.

The free version of PyMol (https://pymol.org) was used for the visualization of the interface of interaction, the visualization of hot spots and polymorphic residues. Hot spots residues were identified by the KFC2 server. KFC2 evaluates biochemical environment around each residue in the complex structure and compares with known hot spots already determined experimentally. The prediction is based on two main parameters, the first one related to a conformation specificity (K-FADE) and the second to biochemical features (K-CON) [24, 25]. Finally, the polymorphic residues were identified through the dbSNP (data base of single nucleotide polymorphism; https://www.

ncbi.nlm.nih.gov/SNP).

RESULTS AND DISCUSSION

TFF3 interacts with PAR-2 receptors and regulate inflammation processes

The amino acid sequence of the protein TFF3 was retrieved from the National Center for Biotechnology Information (NCBI) in order to evaluate possible conserved domains. TFF3 contains a cysteine-rich domain of 45 amino acid residues where six cysteines are linked by three disulphide bonds [26-29]. The domain enables the protein to be active and to interact with protein partners. The name of the protein is related to the TFF domain, which comprises three loop regions, the so-called trefoil region [29, 30]. TFF3 is expressed in several tissues. The protein has a role in cell growth, cell differentiation and tissue recovery as mucosal wound healing [31, 32]. This protein is upregulated in several types of cancers [33,34], including endometrial cancer [35, 36], gastroadenocarcinoma [37]. TFF3 is involved in inflammatory-related processes and immune response [38]. As mentioned before, endometriosis is an estrogen-dependent disease featured by intensive inflammatory processes, TFF3 might have a role in endometrial tissue remodeling in women at reproductive age. It has been shown that endometriosis patients has higher levels of TFF3 in the peritoneal fluid [38]. The TFF3 activity is guaranteed by its conformational structure and PPIs. Alterations within the TFF3 gene coding region may interfere with its functions and protein-interaction patterns in a way that it could lead to an increased susceptibility to endometriosis.

TFF3 interacts with and regulates the protease activated receptor 2 (PAR-2) activation in human tissues [39] (Figure 1). PAR-2 is related to inflammatory responses, overweight [40], metabolism [41] and respond to proteolytic enzymes from microorganisms [42]. Figure 2 shows amino acid residues that contribute to the interaction between TFF3 and PAR-2. All residues displayed are separated from the binding protein by no more than 4Å. Figure 3 shows a different view of the interaction, highlighting the interface of interaction between TFF3 and PAR-2.

Overexpression of TFF3 contributes to the malignant progression in cancer cells [43]. Similar process occur in endometriosis, since TFF3 is able to stimulate the invasion of endometrial cells in tissues away from the uterine cavity by activating the signaling pathways. Several PAR-2 and TFF3 agonist peptides have been shown to downregulate TFF3 expression, which could be used as an alternative therapeutic method to reduce endometriosis lesions, inflammation and pain.

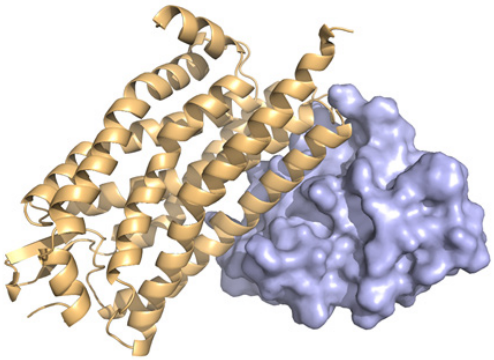

Figure 1: Model for the interaction between TFF3 and PAR-2. The illustration shows the most energetically favorable mode of interaction of TFF3 (blue) and PAR-2 (orange).

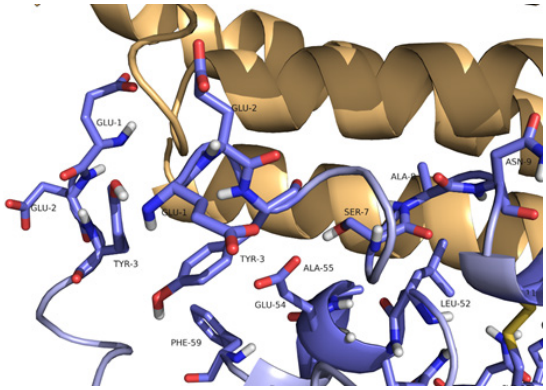

Figure 2: Amino acid residues involved in TFF3-PAR-2 interaction. The TFF3 amino acids represented here (blue chain) are less than 4Å away than PAR-2 (orange chain) and contribute significantly to the conformational structure of the protein complex.

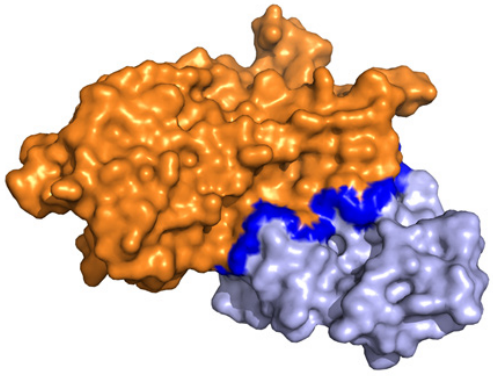

Figure 3: TFF3-PAR-2 interface of interaction. The figure highlights the interface of interaction between TFF3 and PAR-2. The interaction is important for the regulation of cytokine levels in normal conditions. Endometriosis patients show overexpression of TFF3 and consequently increased inflammation processes. The inhibition of the TFF3-PAR-2 interaction may reduce TFF3 activity, inflammation and pain.

Identification of hot spot residues on the interface of interaction between TFF3 and PAR-2

Hot spots are amino acid residues accounting for a significant amount of the binding free-energy between interacting proteins [44]. They maintain the energetic stability of the protein

complex, thus they are related to specific functions performed by the protein or protein complex [45]. Hot spots regions are determined by mutagenesis experiments. Bioinformatic analyses rely on these experimental data and predict hot spots with high accuracy [24,25]. We found 16 hot spots on the interface of interaction between TFF3 and PAR-2 (data not shown). The most energetically favorable hot spots amino acids are represented in figure 4. Among those residues, nine were on TFF3 surface and 7 on the PAR-2 surface. These amino acid residues are highly conserved among species even though there may be certain variation among the human population. This genetic variation may result in a higher susceptibility to endometriosis development showed by certain patients.

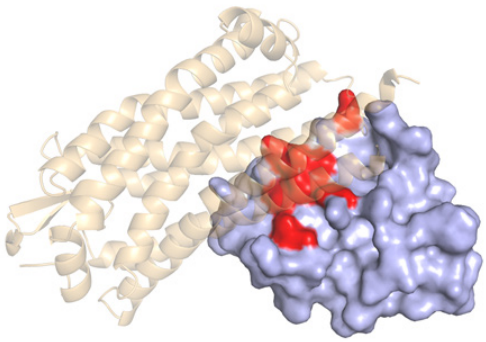

Figure 4: Hot spots residues on TFF3 surface. Certain residues contribute to the conformation state of the TFF3-PAR-2 complex, they are called hot spots (red). Genetic variation affecting hot spot residues may interfere with the activity of proteins and cause variations in the normal concentration of such proteins in cells leading to disease susceptibility. Orange – PAR-2; Blue – TFF3.

We found four polymorphic residues out of nine hot spots residues on the TFF3 surface (Table 1). These SNPs might reflect an increased susceptibility to endometriosis due to alteration in the conformational structure of the protein, alteration of the interaction partners and consequently anomaly protein activity [46]. It is possibly that polymorphic amino acid residues alter the ability of the protein to control inflammation and the invasion of endometrial cells into other tissues. Moreover, SNPs in hot spot residues may also be related to overexpression of TFF3 in patients with endometriosis.

Table 1: Hot spot residues on the interface of interaction between TFF3 and PAR-2.

Residue	Non-synonymous polymorphism
GLU 2	-
SER 7	PHE
ALA 8	-
ASN 9	ASP
CYS 11	ARG
ASN 32	SER, LYS
CYS 37	-
LEU 52	-
ALA 55	-

Structure-based design of TFF3-PAR2 inhibitor peptides as an alternative method for endometriosis treatment

Targeting TFF3 overexpression using structure-based peptide inhibitor may provide a potential therapeutic option for the treatment of endometriosis in the future. We designed several peptides based on the interface of interaction between TFF3 and PAR-2. As discussed above, overexpression of TFF3 is related to inflammation and adhesion of endometrial cells in ectopic tissues and organs. PAR-2 plays important roles regarding inflammation and regulating TFF3 levels. Several approaches have been tested and mostly the focus is the inhibition of TFF3 and PAR-2 interaction. We hypothesize that inhibiting TFF3 activity when this protein is overexpressed would also reduce inflammation, adhesion of endometrial cells and pain. Figure 5 show the most energetically favorable peptides biding to hot spot amino acid residues on the surface of TFF3. The rational design of peptide is a promising approach for new options of endometriosis treatment, in a way that it could increase the quality of life of patients.

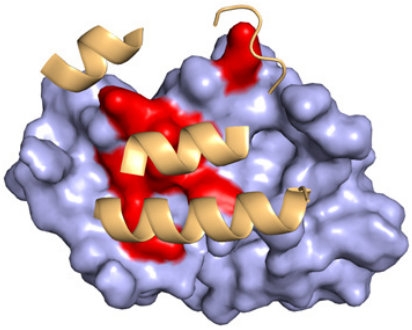

Figure 5: TFF3-PAR-2 structure-based inhibitor peptides. Peptides were designed based on the interface of interaction between TFF3 and PAR-2. Such peptides inhibit the interaction and the excessive activity of TFF3 that is overexpressed in endometriosis patients.

CONCLUDING REMARKS

There is a very high number of women with chronic pelvic pain and infertility related to endometriosis. The disease brings high cost to health care systems worldwide. The present in silico approach provide important insights to the development of more effective treatment options for endometriosis, which would improve quality of life of patients and increase reproductive potential in women affected by the disease. Here, we identified hot spot residues on the interface of interaction between TFF3 and PAR-2, both proteins related to inflammation and immune system. Moreover, TFF3 is known to be overex-

pressed in endometriosis and other diseases such as cancer. We designed structure-base peptides that intend to reduce the activity of TFF3 when this protein is overexpressed. The limitation of the present study relies on the fact that each peptide should be tested and validated; the procedures can be time consuming and expensive. We believe that inhibitor peptides could be a promising new approach of treatment for endometriosis in the future.

REFERENCES

1. Vercellini P, Viganò P, Somigliana E and Fedele L. (2014). Endometriosis: pathogenesis and treatment. Nat Rev Endocrinol. 10(5): 261-275.
2. Macer ML and Taylor HS. (2012). Endometriosis and infertility: a review of the pathogenesis and treatment of endometriosis-associated infertility. Obstet Gynecol Clin North Am. 39(4): 535-549.
3. Rodgers AK and Falcone T. (2008). Treatment strategies for endometriosis. Expert Opin Pharmacother. 9(2): 243-255.
4. Dowlut-McElroy T and Strickland JL. (2017). Endometriosis in adolescents. Curr Opin Obstet Gynecol. 29(5): 306-309.
5. Ahn SH, Singh V and Tayade C. (2017). Biomarkers in endometriosis: challenges and opportunities. Fertil Steril. 107(3): 523-532.
6. Kavoussi SK, Lim CS, Skinner BD, Lebovic DI, et al. (2016). New paradigms in the diagnosis and management of endometriosis. Curr Opin Obstet Gynecol. 28(4): 267-276.
7. Abrao MS, Gonçalves MO da C, Dias JA, Podgaec S, et al. (2007). Comparison between clinical examination, transvaginal sonography and magnetic resonance imaging for the diagnosis of deep endometriosis. Hum Reprod. 22(12): 3092-3097.
8. Zarghi A and Arfaei S. (2011). Selective COX-2 Inhibitors: A Review of Their Structure-Activity Relationships. Iran J Pharm Res. 10(4): 655-683.
9. Fan W, Huang Z, Xiao Z, Li S, et al. (2016). The cytochrome P4501A1 gene polymorphisms and endometriosis: a meta-analysis. J Assist Reprod Genet. 33(10): 1373-1383.
10. 10. Matalliotakis M, Zervou MI, Matalliotaki C, Rahmioglu N, et al. (2017). The role of gene polymorphisms in endometriosis. Mol Med Rep. 16(5): 5881586.
11. Gaetje R, Holtrich U, Engels K, Kissler S, et al. (2007). Endometriosis may be generated by mimicking the ontogenetic development of the female genital tract. Fertil Steril. 87(3): 651-656.
12. De Conto E, Matte Ú, Bilibio JP, Genro VK, et al. (2017). Endometriosis-associated infertility: GDF-9, AMH, and AMHR2 genes polymorphisms. J Assist Reprod Genet. 34(12): 1667-1672.
13. Matalliotakis M, Zervou MI, Eliopoulos E, Matalliotaki C, et al. (2018). The role of IL-16 gene polymorphisms in endometriosis. Int J Mol Med. 41(3): 1469-1476.
14. Zhao W, Li Y, Zhao J and Kang S. (2018). A functional promoter polymorphism in interleukin 12B gene is associated with an increased risk of ovarian endometriosis. Gene. 666: 27-31.
15. Fayez D, Saliminejad K, Irani S, Kamali K, et al. (2018). Arylamine N-acetyltransferase 2 Polymorphisms and the Risk of Endometriosis. Avicenna Journal of Medical Biotechnology. 10(3): 163-167.
16. Barbosa AM, de Souza SR, Frare AB, Costa E Silva RC, et al. (2016). Association of CYP1A1 (cytochrome P450) MspI polymorphism in women with endometriosis. Genet Mol Res. 15(3).
17. Silva KSF and Moura KKVO. (2016). Genetic polymorphisms in patients with endometriosis: an analytical study in Goiânia (Central West of Brazil). Genet Mol Res. 15(2).
18. Santos TR, Silva KSF e, Silva RCPC, Moura KKVO, et al. (2018). Research Article Infertility caused by an association between Arg72Pro polymorphism of the p53 gene and Glu298Asp of the eNOS gene in patients with endometriosis. Genet Mol Res. 17(3).
19. de Morais MP, Curado RF, E Silva KSF, Moura KKVO, et al. (2016). Male idiopathic infertility and the TP53 polymorphism in codon 72. Genet Mol Res. 15(4).
20. Linzhi Y, Dan C, Arhin SK, Ledan W, et al. (2018). Screening for novel peptides specifically binding to the surface of ectopic endometrium cells by phage display. Cell Mol Biol. 64(11): 36-40.
21. Ghoorah AW, Devignes M-D, Smaïl-Tabbone M and Ritchie DW. (2016). Classification and Exploration of 3D Protein Domain Interactions Using Kbdock. Methods Mol Biol. 1415: 91-105.
22. Tovchigrechko A and Vakser IA. (2006). GRAMM-X public web server for protein-protein docking. Nucleic Acids Res. 34(Web Server issue): W310-314.
23. Tovchigrechko A and Vakser IA. (2005). Development and testing of an automated approach to protein docking. Proteins. 60(2): 296-301.
24. Darnell SJ, LeGault L and Mitchell JC. (2008). KFC Server: interactive forecasting of protein interaction hot spots. Nucleic Acids Res. 36.
25. Darnell SJ, Page D and Mitchell JC. (2007). An automated decision-tree approach to predicting protein interaction hot spots. Proteins. 68(4): 813-823.
26. Otto B and Wright N. (1994). Trefoil peptides. Coming up clover. Curr Biol. 4(9): 835-838.

27. Bork P. (1993). A trefoil domain in the major rabbit zona pellucida protein. Protein Sci. abril de. 2(4): 669-670.
28. Hoffmann W and Hauser F. (1993). The P-domain or trefoil motif: a role in renewal and pathology of mucous epithelia? Trends Biochem Sci. 18(7): 239-243.
29. Tan XD, Hsueh W, Chang H, Wei KR, et al. (1997). Characterization of a putative receptor for intestinal trefoil factor in rat small intestine: identification by in situ binding and ligand blotting. Biochem Biophys Res Commun. 237(3): 673-677.
30. Finn RD, Attwood TK, Babbitt PC, Bateman A, et al. (2017). InterPro in 2017-beyond protein family and domain annotations. Nucleic Acids Res. 45(D1): D190-199.
31. Jiang G, Zhong X, Cui Y, Liu W, et al. (2010). IL-6/STAT3/TFF3 signaling regulates human biliary epithelial cell migration and wound healing in vitro. Mol Biol Rep. 37(8): 3813-3818.
32. Ge H, Gardner J, Wu X, Rulifson I, et al. (2015). Trefoil Factor 3 (TFF3) Is Regulated by Food Intake, Improves Glucose Tolerance and Induces Mucinous Metaplasia. PLOS ONE. 10(6): e0126924.
33. Choi B, Lee H-J, Min J, Choe H-N, et al. (2017). Plasma expression of the intestinal metaplasia markers CDH17 and TFF3 in patients with gastric cancer. Cancer Biomark. 19(3): 231-239.
34. Nørgaard M, Haldrup C, Storebjerg TM, Vestergaard EM, et al. (2017). Comprehensive Evaluation of TFF3 Promoter Hypomethylation and Molecular Biomarker Potential for Prostate Cancer Diagnosis and Prognosis. Int J Mol Sci. 18(9).
35. Mhawech-Fauceglia P, Wang D, Samrao D, Liu S, et al. (2013). Trefoil factor family 3 (TFF3) expression and its interaction with estrogen receptor (ER) in endometrial adenocarcinoma. Gynecol Oncol. 130(1): 174-180.
36. Neubert D, Ondrová D, Hambálek J, Maděrka M, et al. (2018). [Elevated levels of TFF3 in endometrial cancer patients]. Ceska Gynekol. 83(2): 109-114.
37. Xu C, Yue L, Wei H, Zhao W, et al. (2013). Significance of TFF3 protein and Her-2/neu status in patients with gastric adenocarcinoma. Pathol Res Pract. 209(8): 479-85.
38. Henze D, Doecke W-D, Hornung D, Agueusop I, et al. (2017). Endometriosis Leads to an Increased Trefoil Factor 3 Concentration in the Peritoneal Cavity but Does Not Alter Systemic Levels. Reprod Sci. 24(2): 258-267.
39. Barrera GJ and Tortolero GS. (2016). Trefoil factor 3 (TFF3) from human breast milk activates PAR-2 receptors, of the intestinal epithelial cells HT-29, regulating cytokines and defensins. Bratisl Lek Listy. 117(6): 332-339.
40. Lim J, Iyer A, Liu L, Suen JY, et al. (2013). Diet-induced obesity, adipose inflammation, and metabolic dysfunction correlating with PAR2 expression are attenuated by PAR2 antagonism. FASEB J. 27(12): 4757-4767.
41. Badeanlou L, Furlan-Freguia C, Yang G, Ruf W, et al. (2011). Tissue factor-protease-activated receptor 2 signaling promotes diet-induced obesity and adipose inflammation. Nat Med. 17(11): 1490-1497.
42. Lee SE, Jeong SK and Lee SH. (2010). Protease and Protease-Activated Receptor-2 Signaling in the Pathogenesis of Atopic Dermatitis. Yonsei Med J. 51(6): 808-822.
43. Yuan Z, Chen D, Chen X, Yang H, et al. (2017). Overexpression of trefoil factor 3 (TFF3) contributes to the malignant progression in cervical cancer cells. Cancer Cell International. 17(1): 7.
44. Clackson T and Wells JA. (1995). A hot spot of binding energy in a hormone-receptor interface. Science. 267(5196): 383-386.
45. Keskin O, Ma B, Nussinov R. (2005). Hot regions in protein--protein interactions: the organization and contribution of structurally conserved hot spot residues. J Mol Biol. 345(5): 1281-1294.
46. Schaefer C and Rost B. (2012). Predict impact of single amino acid change upon protein structure. BMC Genomics. 13(Suppl 4): S4.