Protein-protein Interaction Analysis of Contributing Molecules in Dura mater Healing Process

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Abstract: Background: Dura mater is a special tissue that fulfills a critical function in brain anatomy and physiology. This tissue contains numerous cells, stem cells, and growth factors. This research investigates the protein interaction contributing to dura mater healing process. Methods: We use the available analysis software to perform the protein-protein interaction (PPI) analysis (http://gpsprot.org/index.php). GPS Protein is an interactive platform for visualizing human protein interaction by integrating HIPPIE and CORUM databases. We excluded HIV-1 proteomic and RNAi databases, instead focused on human PPI (Confidence level 0.75). Two proteins were inputted as query to identify the potential protein network in Dura mater healing according to previous studies, i.e. fibroblast growth factor-2 (FGF2) and transforming growth factor beta-1 (TGFβ1). Results: PPI results shows a high level (confidence level > 0.75) of protein-protein interaction of TGFβ1 to 197 other proteins (Confidence level ranges: 0.49 - 0.87), and PPI of FGF2 to 26 other proteins (Confidence level ranges: 0.0-0.97). TGFβ1 interactions showed the important interactions to some remodeling proteins. TGFβ1 encoded regulates cell proliferation, differentiation, growth, expression modulation and the activation of other growth factors. It also induces epithelial-to-mesenchymal transition (EMT) and cell migration. Conclusion: This bioinformatics approach is the more efficient and cheaper method for analyzing the molecular aspect of protein that has a special contribution in Dura mater healing process. These results could beneficial in focusing further researches for more complex laboratory examinations.

Keywords: Bioinformatic, dura mater, healing, protein interaction, transforming growth factor beta-1.

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Introduction

Dura mater is a special tissue that fulfills a critical function in brain anatomy and physiology. It is the outer layer of meninges, consisting of collagen fibers, fibroblast, elastic fibers and even the epithelial and mesenchymal stem cells. Dura mater defects caused by trauma, neoplastic invasion, irregular laceration, coagulation shrinkage, manipulation in neurosurgery, prolonged retraction and pathology process (Chiari malformation) hinders Dura mater from closing perfectly. As it serves as mechanical barriers, tear in Dura mater will cause infections, cerebro-spinal fluid leakage, and pseudomeningoele that could lead to increased mortality rate, morbidity rate and health cost.

Dura mater has no ability to regenerate. Using graft’s materials for the secondary closure of the tear in Dura mater is necessary. Since Abbe et al. used the rubber in 1895 to close the Dura mater’s tear, the experts are in pursuit to discover the ideal material to closing the tear in Dura mater. They utilize various sources of autografts (muscle fascia, peritoneum, etc.), allograft (amniotic membrane, lyophilized Dura mater, cadaveric Dura mater), xenograft and other synthetic materials. Each material has its own advantages and disadvantages.

Dural fibroblast and extracellular matrix synthesis are already known as the key substances in Dura mater healing. Some research conducted by Schick et al, Zhong et al, and Goldschmidt et al, outlines the steps of dura mater healing process, involving the fibroblast cells. Another study showed the roles of growth factors contributing to dura mater healing process. FGF-2 and TGFβ1 are also known as the proteins holding a specific role in dura mater healing. FGF-2, by topical application, facilitates Dura mater healing in early phase (3 weeks) and late phase (6 weeks), and TGFβ1 triggers endothelium proliferation and collagen fibers, bone induction, suture fusion and neovascularization process. TGFβ1 serves as a functional growth factor that is only found in mammals, secreted and stored as a latent complex in the extracellular matrix.

Studies on the material graft used for duraplasty in animal models or directly on human require a complex preparation and expensive cost. We need to consider some established results from other research and choose the target proteins or molecules with specific roles on the mechanisms of Dura mater healing process. This research investigates the protein interaction contributing to Dura mater healing process. This step is really important to provide the initial understanding prior to advancing the exploration on the role of specific molecules in complex animal models.

Method

We use bioinformatics resources to identify and predict in silico of the functional protein interaction in Dura mater healing process. We used the available analysis software to perform the protein-protein interaction (PPI) analysis (http://gpsprot.org/index.php). GPS Protein is an interactive platform for visualizing human protein interaction by inputting human integrated protein-protein interaction reference (HIPPIE) and comprehensive resource of mammalian protein complexes (CORUM) databases. We excluded HIV-1 proteomic and RNAi databases, instead focused on human PPI (Confidence level 0.75, high level). Two proteins were inputted as a query to identify the potential protein network in Dura mater healing according to previous studies, i.e. fibroblast growth factor-2 (FGF2) and transforming growth factor beta-1 (TGFβ1) (Table.1).

Profile review on the protein refers to the information available at UNIPROT (https://www.uniprot.org) description, OMIM (https://www.omim.org), and other related official protein data base resources and literature reviewed.
### Table 1. The role of Important Molecule in dura mater healing process showed by previous study

| Molecule | Study (Ref) | Method of the study | Results/Conclusion |
|----------|-------------|---------------------|--------------------|
| TGFβ1    | Zhong et al, 2012 [12] | Experimental, animal model (minipigs) | Improve healing by shortening wound healing time and improve tensile strength of the dura mater possibly by facilitating fibroblast proliferation and differentiation, accelerating collagen synthesis, and inhibiting collagen breakdown. TGFβ1 was also involved in up-regulating the angiogenic growth factor vascular endothelial growth factor which may also be a way to accelerate healing. |
|          | Yokogawa et al, 2015 [15] | Experimental, animal study (mice) | as a master switch in inducing, forming, and maintaining fibrosis. |
| bFGF/FGF-2 | Zhong et al, 2012 [12] | Experimental, animal model (minipigs) | Improve healing by shortening wound healing time. The mechanism of bFGF promotion of tissue healing may be facilitation of migration and proliferation of fibroblasts and endothelial cells, which contributes to the formation of vessels. |
|          | Nurata et al, 2009 [13] | Experimental, animal model (rats) | FGF-2 facilitates dura mater healing in both the early and late phases of wound healing. The quality of the dura mater healing remains time-dependent because there was also a significant difference between the FGF-2 groups after 3 and 6 weeks. |

### Result

Our results shows a high level (confidence level > 0.75) of protein-protein interaction of TGFβ1 to 197 other proteins (Confidence level ranges: 0.49 - 0.87, figure-1), and PPI of FGF2 to 26 other proteins (Confidence level ranges: 0.0-0.97, figure-2). PPI of TGFβ1 specifically interacts with several proteins involved in remodeling process. PPI analysis shows the TGFβ1 interaction to SMAD3, COL2A1, MMP2, ENG, DCN, LAMB1, and FSTL1 (table 2). There are no PPI of FGF2 to other remodeling proteins.
Figure 1: The results of PPI connection of TGFβ1

Figure 2: The results of PPI connection of FGF2
Interaction of TGFβ to others protein correlates to its physiology functions in which it signals the system of its main function. The interaction between TGFβ1 and SMAD3 as well as ENG proteins shows the signaling function in their interactions. Some other protein interactions relates to the regulation of the final function on target cellular tissue. PPI of TGFβ1 to COL2A1, MMP2, and LAMB1 relates to its functions in controlling cell proliferation, differentiation, and migration. The complex regulation resulted from the interaction impacts the process of remodeling, tissue repairing, and tissue development. The interaction of TGFβ1 to DCN and FSTL1 serves in specific intermediate function, such as fibril formation (DCN) and other growth factor modulation (FSTL1).

The PPI of TGFβ1 in relation to the specific high confidence score of remodeling proteins are the gateway to performing complex investigation on Dura mater healing process in complex animal model or on human (Table 2).

Table 2. Score Results of PPI analysis and Description of the Protein That Interact to TGFβ1

| Gene A (Uniprot Code) | Gene B (Uniprot Code) | Score | Protein B Name | Cellular Location Protein B | Function of Protein B |
|-----------------------|-----------------------|-------|----------------|---------------------------|-----------------------|
| SMAD3 (P84022)        | mothers against decapentaplegic homolog 3 | 0.75   | Nucleus and cytoplasms | -Intracellular signal transducer and transcriptional modulator activated by TGFβ. | -An inhibitory effect on wound healing. |
| COL2A1 (P02458)       | collagen alpha-1(II) chain | 0.85   | Extracellular region or secreted | -Type II collagen is specific for cartilaginous tissues. | -Essential for the normal embryonic development of the skeleton, for linear growth and for the ability of cartilage to resist compressive forces. |
| MMP2 (P08253)         | 72 kDa type IV collagenase | 0.81   | Extracellular matrix, nucleus, membrane and mitochondrion | -Ubiquitinous metalloproteinase that involved in diverse functions such as remodeling of the vasculature, angiogenesis, tissue repair, tumor invasion, inflammation, and atherosclerotic plaque rupture. |
| TGFβ1 (P01137)        | mothers against decapentaplegic homolog 3 | 0.85   | Plasma membrane | -Acts as TGF-beta coreceptor and is involved in the TGF-beta/BMP signaling cascade. | -Vascular endothelium glycoprotein that plays an important role in the regulation of angiogenesis. -Regulates the migration of vascular endothelial cells. |
| ENG (P17813)          | endoglin | 0.85   | Extracellular matrix | May affect the rate of fibrils formation |
| DCN (P07585)          | decorin | 0.87   | Extracellular matrix | -Laminin subunit beta-1 |
| LAMB1 (P07942)        | laminin subunit beta-1 | 0.82   | Basement membrane | -Binding to cells via a high affinity receptor, laminin is thought to mediate the attachment, migration and organization of cells into tissues during embryonic development by interacting with other extracellular matrix components. -Involved in the organization of the laminar architecture of cerebral cortex. |
| FSTL1 (Q12841)        | follistatin-related protein 1 | 0.76   | Extracellular region or secreted | May modulate the action of some growth factors on cell proliferation and differentiation. |

*Function description based on the [https://www.uniprot.org/uniprot/](https://www.uniprot.org/uniprot/)*
TGFβ gene is located in chromosome 19q13.1- q13.3 in human. Its precursor gene contains 7 exons and very large introns. This protein contains 391 amino acids, and 112 C-terminal amino acids that will progress to mature proteins. The proteolysis site is located at Arg - Arg dipeptide residues. The gene precursor also contains 3 potential N-glycosylation sites. Non-reduced purified TGFβ protein from human blood platelets has a molecular mass of 25 kD. Under reduced conditions, it migrates with an apparent molecular mass of 12.5 kD, indicating that TGFβ consists of two polypeptide chains linked by intermolecular disulfide bridges. The gene can isolate in all solid tumors from all ectoderm, mesoderm and endoderm origins. Using the Nothern blot analysis, we can detect about 2.5-kb of proteins. The transcript of TGFβ1 can also be found in normal lymphocytes of normal peripheral blood, platelets and in placenta. The gene will not be found in liver cells, except in a hepatoma cells line.

Many cells synthesize TGFβ1 and have specific receptors for this peptide. High levels of TGFβ1 mRNA and/or protein have been found in developing cartilages, skin, endochondral and membrane bones. These preferred location indicates the role of proteins in growth and differentiation process of the tissue. TGFβ1, as the key regulator of the brain's responses to injury and inflammation, has been involved in amyloid-beta deposition in vivo. Another research performed by Wyss-Coray et al[17], found increasing expression of TGFβ1 in astrocytic cells of aged transgenic mice, which indicated the increased deposition of the beta-amyloid precursor protein (APP) in cerebral blood vessels and meninges.

TGFβ is a multifunctional peptide regulating the controls of cell proliferation, differentiation, apoptosis, migration and other functions in many cell types. In adult human, TGFβ holds an integral role in maintaining tissue homeostasis. TGFβ acts synergistically with TGFα in inducing transformation. It also serves as a negative autocrine growth factor. Damage regulation on the TGFβ activation and signaling system leads to activate apoptosis cascade.

TGFβ1, TGFβ2 and TGFβ3 all function through the same receptor signaling systems. SMAD proteins mediate TGFβ signaling to regulate cell growth and differentiation. SnoN regulates the negative feedback of TGFβ signaling. SMAD proteins mediate TGFβ signaling to regulate cell growth and differentiation. TGFβ receptors in some cell can be induced by its ligand stimulation. TGFβ actually secreted as a non-active complex and has to stimulate and activated. SnoN protein regulates the negative feedback of TGFβ signaling.

Discussion

Histologically, the dura mater is composed of a rich network of collagen fibers with intermingled fibroblasts and is covered by subdural neuro-epithelium. This architecture raises the question whether additional cellular outspread from the margins of the Dura tear is involved as a mechanism in wound healing following a duraplasty. Human Dura mater consists of periosteal layer and meningeal layer (inner). The periosteal layers contain osteoprogenitors cells, fibroblast, collagen bundles and blood vessels, while meningeal layers contain fibroblast, elastic fibers, fine collagen fibers and small blood vessels.

There are some key components that could be involved in Dura mater healing process following a tear. Cellular components, scaffolding and growth factors may contribute in the process as some experts has tried to find the proof of cellular regeneration capability of Dura mater. The closing of the Dura mater tear has to be achieved as soon as possible to ensure its physiological function. The utilization of some materials (auto-, allo- xeno- or synthetic grafts) has to achieve the ideal criteria for Dura mater replacements.

Cellular proliferation after Dura tear repair is attributable to stable graft placement, which is one of the key concerns in achieving perfect Dural closure. Fibroblasts are preferred for the wound-healing process because they contribute to the process of graft remodeling, which results in a perfect and stable Dural tear repair. Dura mater cultured on collagen sheeted wells shows migration of cells into the central tear after a period of 2–5 days. The cells of fibroblast has an elongated spindly cell appearance, which transforms into an elliptical appearance when there is a confluent growth. The results of the research by Schick et. al. indicates the presence of cellular migration from the Dural tear margin as one mechanism in the process of wound healing following a duraplasty. Fibroblasts are detected in the remodeled Dura transplants in animal studies. Collagen-based graft used in almost all animal models shows the same results, confirming its role as the medium for fibroblast cells migration into the tear in dura mater.
Fibroblasts also show the ability to produce new collagen, but the amount of collagen production should not be overestimated as the collagen deposition around the fibroblasts yields in the feedback the inhibition of collagen synthesis and fibroblast replication. In recent study by Goldschmidt et al., they found a contradictory results in human, indicating that fibroblast cells of Dura mater do not need the collagen as growth medium, unlike in animal models. Fibroblast migrates to the tear and the collagen serves as the medium for migration in animal models (minipigs and rabbits). It requires approximately seven days to cover the tear of Dura mater and the collagen acts as a chemotactic for fibroblast. These results confirm the roles of native cells in Dura mater healing process. Our previous study shows the positive expression of Dura mater CD24, CK18, CD73, CD90 and CD105. CD24 and CK18 are epithelial marker and CD73, CD90, CD105 are the markers for mesenchymal stem cells. The roles of all these cells in Dura mater healing process will be further elucidated in our future animal models research. On the other hand, other research use amniotic membrane as the biological material as it contains rich mesenchymal stem cells components, which also shows the roles of fibrocyte and fibroblast in the closing of the brain dura mater tear.

There are two proteins acting as the promoters for natural dura mater tear closure, which are fibroblast growth factor-2 and transforming growth factor beta-1. Exogenous addition of TGFβ1 promotes wound healing without any signs of tissue hyperplasia. This may be related to the normal reactivity of the tissue as adjusted by the local microenvironment and the controlled release of growth factors. TGFβ1 also holds the important roles in vascular remodeling and exerts a biphasic effect on angiogenesis. There is an immunohistochemical expression of TGFβ1 in the dura mater, and another research shows the expression of TGFβ isoform from the dura mater’s cells.

Matrix metallo-proteases (MMPs) are the group of proteolytic enzymes located in extracellular matrix with a specific roles of enabling the process of cells migration and invasion. MMPs can also promote apoptosis by cleaving the laminin, influencing integrin signaling system. Specifically, MMP-2 (gelatinases) has a role in tumor vasculature where it acts as positive and negative regulator of angiogenesis process. MMPs also promote epithelial-mesenchymal transitions (EMT) by proteolytic activation of TGFβ. EMT occurs during tissue repair and is regulated by TGF-β1 via activation of Erk signaling pathway. A recent study demonstrates that EMT generates cells with stem-like properties, which may be beneficial during tissue repair. TGF-β1 also increases the expression of MMP-2 and MMP-9 in the airway epithelial cells and other results show elevated MMP-2 and MMP-9 levels associated with fibroblasts, during the angiogenic phase in early human dermal wound healing. In the process of migration (metastase) and invasion of tumors cells, the activation of TGFβ1 is involved in proteolytic cleavage of latency-associated peptide (LAP) by soluble MMP-2. Wound healing is dependent on and regulated by complex interactions of different cells types, growth factors and their receptors, integrin, chemokine, cytokines, MMPs, tissue inhibitors of metalloproteinase, proprotein convertase and extracellular matrix components.

Following tissue injury, platelets will aggregate at the site of injury, activated by binding negatively charged of extravascular tissue, which then de-granulate and release some growth factors, such as TGFβ. Endothelial vasculogenesis or angiogenesis is initiated during the stimulation and recruitment of monocytes, neutrophil and macrophage. This process initiates the inflammatory phase of wound healing. During the inflammatory phase, the monocytes subsequently differentiate into macrophages, secreting MMPs to remove debris at wound site and promote healing process. Neutrophils and macrophages also release TGFβ and platelet-derived growth factor (PDGF), which activate fibroblast and initiate the proliferative phase. Fibroblast infiltrates the wound and produces the MMPs in proliferative phase. Fibroblast will align along the borders of the wound, generating contractile force and facilitating the wound closure.

During proliferative phase of wound healing, the core processes, which are fibroblast migration, matrix mineralization, angiogenesis, granulation and epithelialization, always involve the stem cells (mesenchymal stem cells, epidermal, endothelial progenitor), the cells (fibroblast, endothelial cells and keratinocytes), growth factors and cytokines (TGFβ, TGFα, IGF, etc), MMP (MMP-2, 3, 1, 8, 19 etc), tissue inhibitor of metallo-proteinases (TIMP), collagen (type I, II, III etc) and integrin. Transforming growth factor beta is an important growth factor regulating different cellular functions in all phases of wound healing: including extracellular matrix production, protease expression, migration, chemotaxis, differentiation, and proliferation of different cell types.
TGF-β1 is important for inflammatory phase, angiogenesis, granulation tissue formation regulation, extracellular matrix remodeling and is essential for re-epithelialization. In humans, the expression of TGF-β1 and TGF-β2 is increased in wounds by day 7, regulating scar contraction. TGF-β1 modulates differentiation of fibroblasts into myofibroblasts via Rho GTPase activation. Endoglin is a Type III auxiliary coreceptor (TβRIII) for TβRII, ALK1 and activin, such as kinase 5 (ALK5), which interacts with other TGF-β family members. Endoglin is expressed on a number of cell types, including proliferating endothelial cells, activated monocytes, tissue macrophages, stromal cells, pre-B cells, erythroid precursors, syn-cytiotrophoblast, cytотrophoblasts, and tumor cells. TGF-β1 binds to Endoglin by association with the TGF-β type II receptors. It is well known that hypoxic conditions occur during wound healing. Hypoxia controls angiogenic growth factor and cytokine production, in addition to inducing Endoglin expression through the hypoxia-inducible factor-1 (HIF-1) and p38 pathways. Due to the important role that Endoglin plays in a number of cellular processes and the elevated expression during wound healing, Endoglin is likely to have a central role in this process. Endoglin is highly expressed in mesenchymal stem cells (MSCs), but this gradually diminishes with increased differentiation potential, with no expression seen on differentiated cells. Circulating Endoglin which is expressing mesenchymal progenitor cells are also present in normal individuals, and the percentage of MSCs (Endoglin positive, CD44+, CD13+, CD29+, and CD90+) is increased during burn injury, suggesting a role during healing. Fibroblasts play an important role in fibroplasia and granulation tissue formation during wound healing, with significant elevation in fibroblast-associated Endoglin levels as observed between days 4 and 10 of wound healing. Endoglin is expressed in a broad spectrum of proliferating cells and stem cells, regulating important cellular functions that are involved in the wound healing process. Decorin (DCN) and biglycan are the proteoglycans inhibiting active extra-cellular TGFβ (inhibitors of TGFβ) and are associated with other components of elastic fibers (fibrillin), which regulate the expression of fibrillin-1. Less decorin found in stiff skin syndrome (SSS) caused by mutations in glucose transporter (GLUT 10). In the context of healing process, the presence of decorin in tissues can increase macrophage activation. These phagocytic cells play a critical role during inflammation. In the early stages, neutrophils are present at the inflammatory loci, but disappear after 24–48 h. Later, macrophages reach these loci, where they remain until inflammation heals. In the later phases of inflammation, macrophages eliminate non-self structures, remove all debris (including apoptotic bodies), and remodel impaired tissues.

Follistatin-like 1 (FTSL1) is a glycoprotein secreted during the development phase and under the condition of some diseases. FTSL1 is involved in the development of different organogenesis, including early development of the lungs, ureter, CNS and skeleton. This protein interacts with multiple TGFβ superfamily receptors and is identified as a TGFβ inducible gene. This protein is significantly expressed in cardiovascular disease, cancer progression and systemic autoimmune disease. FTSL1 has the roles in the protection and regenerative capability of cardiovascular diseases. Overexpression of FTSL1 and reduced expression of MMP-2 in some cancer decrease the cancer cells ability for migrating and invading other tissue. FTSL1 stimulation also induces the switch of E-cadherin to N-cadherin expression, pointing toward EMT. In other research, it is showed that collagen accumulation reduces in injury of liver and lung due to deficient of FTSL1 expression. The effect mechanism on fibrosis is most probably due to the disruption of TGF-β1/BMP (bone morphogenetic protein) balance by FTSL1 as the capacity of FTSL1 inhibits Smad1/5/8-mediated BMP4 signaling and the stimulation of FTSL1 expression via Smad2/3 mediated TGF-β1 signaling. Overexpression of FTSL1 also reduces reduced expression marker of fibrosis, such as TGFβ1, collagen-I, collagen-III and connective tissue growth factor. This phenomena is undergoing research in nonhuman primate models in order to explain the effect of fibrosis in Dura mater healing process. Our results show that more PPI of TGFβ1 than the other import remodeling proteins. There are some basic results on the crosstalk between TGFβ1 and other remodeling proteins in general. The process of Dura mater healing, specifically involving some remodeling proteins, still yet to be researched on mammal model research.

Conclusion

In the context of Dura mater healing process, we have confirmed that some protein molecules are involved in the process. FGF2 and TGFβ1 contribute to this process. Since the protein-protein interactions show the results of interaction of TGF β1 with others remodeling protein, we can propose other research in vivo to prove the biological process in complex animal models. TGFβ1 is one of the key player in initiation and sustainable of healing process, this specific roles in the aspect of dura mater healing has to investigate. This
A bioinformatics approach is a more efficient and cheaper method for analyzing the molecular aspects of protein that have a special contribution on Dura mater healing process.

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