# Drug discovery in spinal cord injury-induced osteoporosis: a text mining-based study

**Chenfeng Wang[1], Yang Xu[2], Lin Han[1], Weiqing Wu[1], Xuhua Lu[1]**

[1]Department of Orthopaedics, Shanghai Changzheng Hospital, Shanghai, China; [2]Department of Orthopaedics, Xiamen University, Xiamen, China

*Contributions:* (I) Conception and design: C Wang, Y Xu; (II) Administrative support: X Lu; (III) Provision of study materials or patients: C Wang; (IV) Collection and assembly of data: C Wang, L Han, W Wu; (V) Data analysis and interpretation: C Wang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

*Correspondence to:* Xuhua Lu, MD. Department of Orthopaedics, Shanghai Changzheng Hospital, Naval Medical University, No. 415, Feng Yang Street, Huangpu District, Shanghai 200003, China. Email: xuhualu@hotmail.com.

**Background:** Spinal cord injury (SCI) and osteoporosis (OP) are common diseases in spine surgery, and OP could be the complication of SCI. However, SCI-induced OP is a complex pathologic process and drug discovery is limited, which restricts the study in the mechanism and treatment of the disease. This study aims to identify the genes and molecular pathways related to SCI-induced OP through computational tools and public datasets, and to explore drug targeting therapy, ultimately preventing the occurrence of OP after SCI.

**Methods:** In this study, common genes related to SCI and OP were obtained by text mining, then which conducted the functional analysis. Protein-protein interaction (PPI) networks were constructed by STRING online and Cytoscape software. Finally, core genes and potential drugs were performed after undergoing drug-gene interaction analysis which also completed functional analysis.

**Results:** A total of 371 genes common to 'SCI' and 'OP' were identified by text mining. After functional analysis, 207 significant genes were screened out. Subsequently, PPI analysis yielded 23 genes targetable by 13 drugs which were the candidate to treat SCI-induced OP.

**Conclusions:** Taken together, siltuximab, olokizumab, clazakizumab and BAN2401 were first discovered to become the potential drugs for the treatment of SCI-induced OP. Drug discovery using text mining and pathway analysis is a significant way to investigate the pathomechanism of the disease while exploring existing drugs to treat the disease.

**Keywords:** Drug discovery; spinal cord injury (SCI); osteoporosis (OP); text mining

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## Introduction

Osteoporosis (OP) is a systemic bone disease characterized by decreased bone density and quality, destruction of the bone microstructure and increased bone fragility, resulting in fractures. As a complication of spinal cord injury (SCI), OP is mainly due to an imbalance in bone formation and resorption, resulting in rapid reduction in bone minerals and stiffness (1). However, the pathogenesis of SCI-induced OP is such an intricacy that we should not simply regard it as a type of disuse OP, associated with various risks such as hormones, neuron lesion itself, and unloading of the spinal cord (2,3).

With the increase of age, hormone levels decrease gradually, the skeletal muscle system becomes aged, and substances required for bone formation such as vitamin D and calcium ions are also decreased, all of which may induce OP (4). Yet, SCI-induced OP is a unique form of neurogenic OP mainly due to the functional imbalance between osteoblasts and osteoclasts following SCI, which is also affected by the severity of injury, body mass index (BMI) and age (5). In addition, SCI has a deleterious effect on the entire skeleton, with the most severe bone

loss and structural deterioration in the lower extremities followed by the sub-lesional vertebrae (6). This bone loss is characterized by the large reduction in cancellous bone mass within the first few years after SCI with cortical bone loss persisting for more than 10 years (7).

A comprehensive understanding about the mechanism underlying SCI-induced OP is helpful to search for treatment strategies. However, traditional treatments cannot meet the increasing needs of OP patients partly because their therapeutic efficacy is not satisfactory enough. It is therefore to make the greatest efforts to mine the potential pharmacological intervention. In recent years, bisphosphonates have received widespread attention from clinicians, owing to their fantastic function of inhibiting bone resorption and bone loss, thus reducing the risk of osteoporotic fracture (8,9). But unfortunately, bisphosphonates have been shown to slow bone loss following SCI but cannot promote new bone formation (10). Similarly, parathyroid hormone (PTH) is considered unlikely to be involved in the pathogenesis of bone loss after SCI (2). Other than romosozumab, teriparatide and alendronate which are known to play important roles in OP therapy (11-13), many other potential drugs are waiting for discovery.

With the continuous progress and development of bioinformatical technology, the underlying mechanism of large numbers of diseases regulated by genes and molecules can be explored, suggesting that further understanding the process can provide a guideline for better treatment of OP. Luckily, a therapeutic target database that is further enriched with regulatory mechanisms or biochemical classes has been constructed for drug discovery (14). Meanwhile, the model of marginalized denoising has been applied for the drug-target interacting prediction marginalized denoising (15). A study that relied on bioinformatical analysis has disclosed the pharmacological target for treating COVID-19 (16). Although OP is known as a frequent occurrence in SCI patients, there is no effective treatment for preventing the progression of bone loss. It may be possible to use bioinformatical analysis to find new pharmaceuticals for the treatment of OP following SCI (17). Therefore, computer analysis technology is being regarded as a useful tool in drug selection for common diseases, even cancers and influenza. Luckily, text mining of biomedical literature acts as a catalyst to deeply analyze the interacting relationships between genes and possible mechanisms between diseases via pathways while combining with other bioinformatical methods, finally obtaining the candidate medical therapy.

The aim of the present study was to explore the pathology and mechanism of SCI-induced OP, with the help of text mining, Gene functional analysis, protein-protein interaction (PPI) network construction, and drug discovery, ultimately mining the potential medicines targetable for core genes. First, a list of common genes was acquired via the intersection between the term 'spinal cord injury' and 'osteoporosis'. Secondly, the genes were imported into the DAVID and STRING online databases for further screening out hub symbols. Consequently, candidate drugs corresponding to the core genes originated from the results of drug-gene interaction analysis (*Figure 1*). We present the following article in accordance with the STREGA reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-21-6900/rc).

## Methods

### *Text mining*

The pubmed2ensemble (http://pubmed2ensembl.ls.manchester.ac.uk/), an online database, was utilized for text mining, which could search for the genes associated with diseases or searching terms as far as possible. After inputting one concept of 'spinal cord injury' and another concept of 'osteoporosis', two queries were performed in gene lists. Then all of the unique genes were exported from the results of gene-disease. Subsequently, obtaining the intersecting genes was the origin of the study.

### *Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis*

Depending on the DAVID (http://www.david.com), a web-based tool, the intersection between SCI and OP was conducted by GO and KEGG pathways analysis, thus completing annotation and functional process of common genes through integrating multiple sources. Besides, the biological process, one of the most significant GO analyses, was selected as a screening criterion that false discovery rates (FDRs) were less than 0.05 to acquire a unique gene query. In the next step, we used the KEGG pathway analysis to further mine the core genes closely related to the pathology of SCI and OP, which was above the P value cutoff.

### *PPI network*

Through GO and KEGG analysis and filtration, all the

 

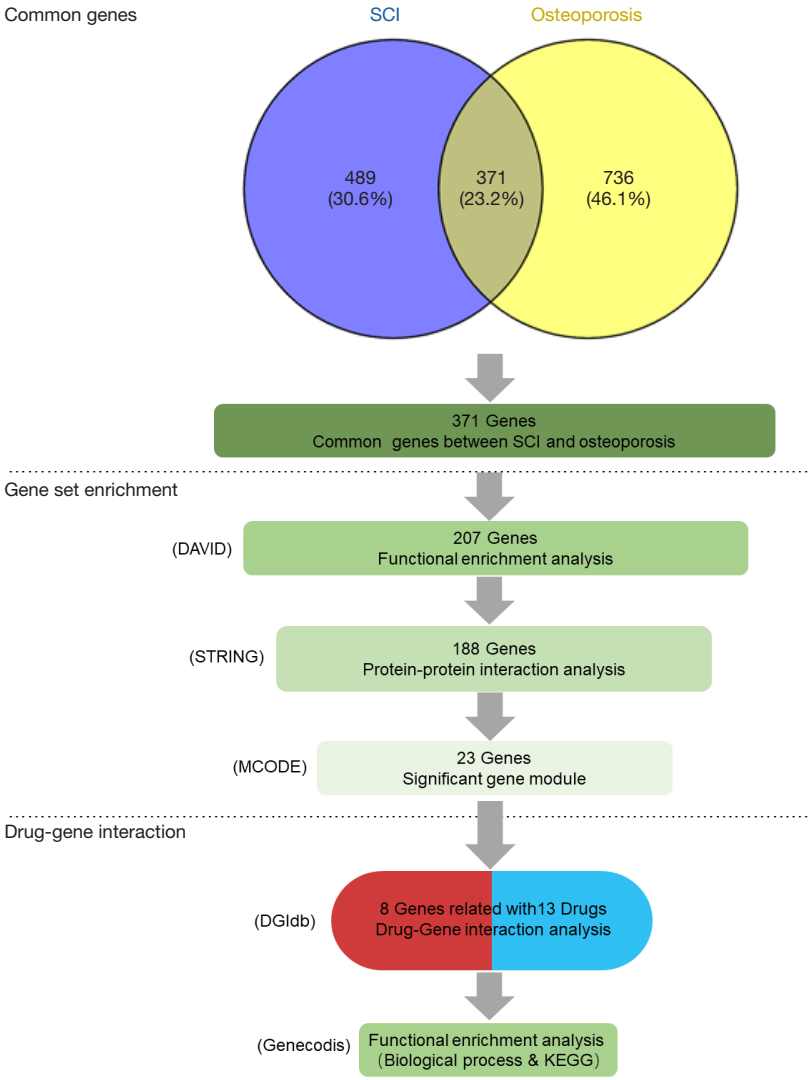


**Figure 1** Summary of overall data mining result. (I) Obtaining common genes: 860 genes were obtained by using the searching term 'spinal cord injury' and 1,107 genes were acquired via the term 'osteoporosis' in pubmed2ensemble, ultimately getting 371 common genes. (II) Gene set enrichment: DAVID functional enrichment analysis was performed using biological process, cellular component, molecular function, and signaling pathways analysis. Subsequently, 23 genes were screened out by using the STRING and Cytoscape software. (III) Drug-gene interaction and functional analysis; 23 genes were imported into the DGIdb and 13 drugs were regarded as the potential medical therapy, while 8 genes were selected as the final genes that completed the functional analysis. KEGG, Kyoto Encyclopedia of Genes and Genomes; SCI, spinal cord injury.

genes were inputted into the STRING (http://string-db.org) online database for investigating the interaction between proteins and constructing their network. Among the high confidence (score 0.900), a 'tsv' file was extracted to obtain significant genes. Importing the file into the Cytoscape software to visualize the network was the first step of cluster analysis. The app of software named Molecular Complex Detection (MCODE) was applied to further build up gene modules and gain hub genes for drug-gene interaction analysis. The cutoff parameters were "degree cutoff =2", "node score cutoff =0.2", "k-core =2", and "max depth =100".

### *Drug-gene interaction and functional analysis*

Hub genes coming from the PPI network and MCODE

modules were imported into the online database, drug-gene interaction, to mine the potential drugs for SCI-induced OP. Under the strict conditions that the drug-gene interacting score was higher than 5 and the type was obvious, final core genes intersecting in SCI and OP were produced for the next functional analysis.

### *Statistical analysis*

The moderate *t*-test was applied to identify differentially expressed genes (DEGs), and Fisher's exact test was used to analyze GO and KEGG enrichment. All statistical analysis was executed in R version 4.0.1 software.

### *Ethical statement*

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

## Results

### *Obtaining common genes of SCI and OP*

After eliminating duplication, the consequences of text mining with conceptions of 'spinal cord injury' and 'osteoporosis' were respectively 860 and 1,108 unique genes. A total of 371 symbols were intersecting between SCI and OP, which would be utilized for the next analysis.

### *GO and KEGG pathway analysis*

GO analysis consists of biological process, cellular component, and molecular function, and the biological process therein is most meaningful. The five most significantly enriched biological process annotations were (I) cell proliferation (FDR =2.86E–55); (II) regulation of cell proliferation (FDR =2.82E–54); (III) positive regulation of multicellular organismal process (FDR =7.42E–53); (IV) response to an organic substance (FDR =4.53E–52); and (V) response to external stimulus (FDR =7.34E–52), containing 354 non-duplicating genes altogether. When it comes to cellular component: (I) extracellular space (FDR =3.86E–38); (II) extracellular region part (FDR =5.50E–28); (III) extracellular region (FDR =3.08E–27); (IV) cell surface (FDR =6.81E–23); and (V) vesicle lumen (FDR =1.43E–18) were the top five of cellular component annotations. As for the molecular function: (I) receptor binding (FDR =2.81E–43); (II) cytokine receptor binding (FDR =9.60E–19); (III) cytokine activity (FDR =2.23E–18); (IV) growth factor activity (FDR =7.33E–16); and (V) hormone activity (FDR =3.70E–15) play an important role in the development of SCI and OP.

KEGG pathway analysis, a method to further identify the significant genes, was including 207 genes common to that of biological process. The results of the ten most enriched KEGG pathway analysis were (I) cytokine-cytokine receptor interaction (FDR =7.01E–15); (II) tumour necrosis factor (TNF) signaling pathway (FDR =7.19E–13); (III) hypoxia-inducible factor-1 (HIF-1) signaling pathway (FDR =1.51E–11); (IV) phosphatidylinositol 3-kinase (PI3K)-Akt signaling pathway (FDR =7.02E–10), and (V) osteoclast differentiation (FDR =6.86E–09), implying the potential mechanism of SCI-induced OP (*Figure 2*).

### *Module screening of PPI network*

In total, 207 genes were imported into the STRING online database and then exported into a file, ultimately analyzed by the Cytoscape. Under the strict controls of condition (high medium confidence, score >0.9), a sum of 188 genes was participating in the construction of the PPI network. Subsequently, the network was uploaded into Cytoscape for cluster analysis by the MCODE app. There are two most significant modules produced by the app. The first module consisted of 15 genes/nodes and 105 edges, while the second module was constructed by 8 genes/nodes and 28 edges (*Figure 3*). In order to mine the candidate drugs for SCI-induced OP, a list of 23 hub genes via adding up the number of genes in the two models was input into the DGIdb online database for drug-gene interaction.

### *Potential therapeutics and functional analysis*

Meeting the screening criteria that the interacting score should be higher than 5 and the type was definite was necessary to investigate the medical therapy. Consequently, 13 drugs (BAN2401, TB-402, drotrecogin alfa, rilotumumab, ficlatuzumab, dusigitumab, siltuximab, olokizumab, clazakizumab, lerdelimumab, fresolimumab, ranibizumab, caplacizumab) corresponding to 8 core genes [amyloid beta precursor protein (*APP*), coagulation factor VIII (*F8*), hepatocyte growth factor (*HGF*), insulin like growth factor 1 (*IGF1*), interleukin 6 (*IL-6*), transforming growth factor beta 2 (*TGFB2*), von Willebrand factor (*VWF*), vascular endothelial growth factor A (*VEGFA*)] were discovered to affect OP (*Table 1* and *Figure 4*). Finally,

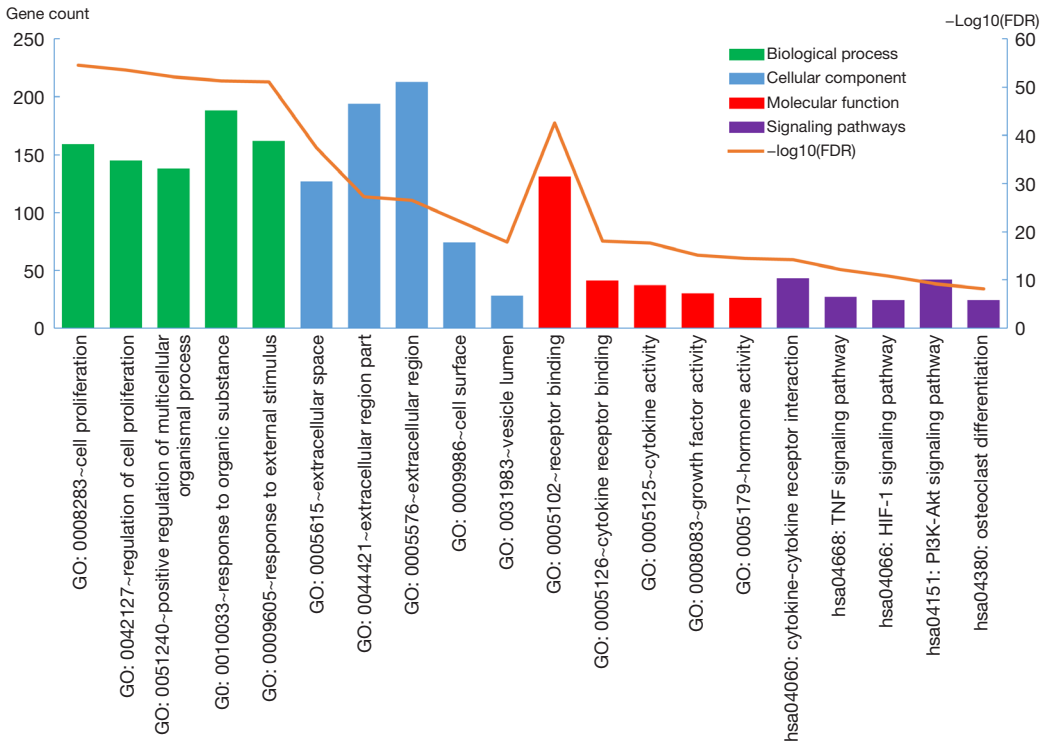


**Figure 2** Gene ontology and KEGG pathway analysis. Biological processes, cellular components, and molecular functions consisted of GO analysis. Green bar charts represented the biological process, blue bar charts represented the cellular component, red bar charts represented the molecular function, purple bar charts represented the signaling pathways, and orange line chart represents –log10 (FDR). FDR, false discovery rate; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

functional analysis of the 8 core genes completed in the Genecodis showed that the top five biological processes were platelet degranulation (FDR =4.25E−16), positive regulation of peptidyl-tyrosine phosphorylation (FDR =8.32E−11), positive regulation of PI3K signaling (FDR =5.43E−08), positive regulation of mitogen-activated protein kinase (MAPK) cascade (FDR =1.24E−07), and positive chemotaxis (FDR =1.78E−06), while the top five KEGG pathways were PI3K-Akt signaling pathway (FDR =3.07E−09), MAPK signaling pathway (FDR =1.03E−07), HIF-1 signaling pathway (FDR =7.57E−07), FoxO signaling pathway (FDR =1.44E−06), and Ras signaling pathway (FDR =5.11E−06), could be seen in *Table 2*.

## Discussion

OP is a major clinical problem associated with many risk factors and etiologies. Surprisingly, SCI is actually numbered among the causes of OP. It is therefore of great clinical significance to clarify the underlying, mine the medical target and select candidate drugs for the sake of the prevention and treatment of SCI-induced OP. The study aimed to realize the drug discovery for SCI-induced OP and ultimately decrease the risk and incidence of OP following SCI. We first obtained common genes between SCI and OP coming from the pubmed2ensemble, and then conducted GO and KEGG pathways analysis to screen out core genes that participated in the construction of the PPI network to further screen out hub genes combined with the MCODE analysis. Finally, these hub genes were imported into the online database DGIdb to acquire related drugs.

According to the criteria, the final 8 genes were screened out, which were associated with 13 drugs and 5 pathways. *APP* was reported as a potential biomarker of OP for drug targets (18), and may also be a promising agent for osteoporotic therapy owing to its role in enhancing receptor activator of NF-κB ligand (RANKL)-induced osteoclast activation (19,20). Besides, alendronate, which is known as an *APP*-targeted medicine with anti-OP and neuroprotection activities, is also applied for OP treatment

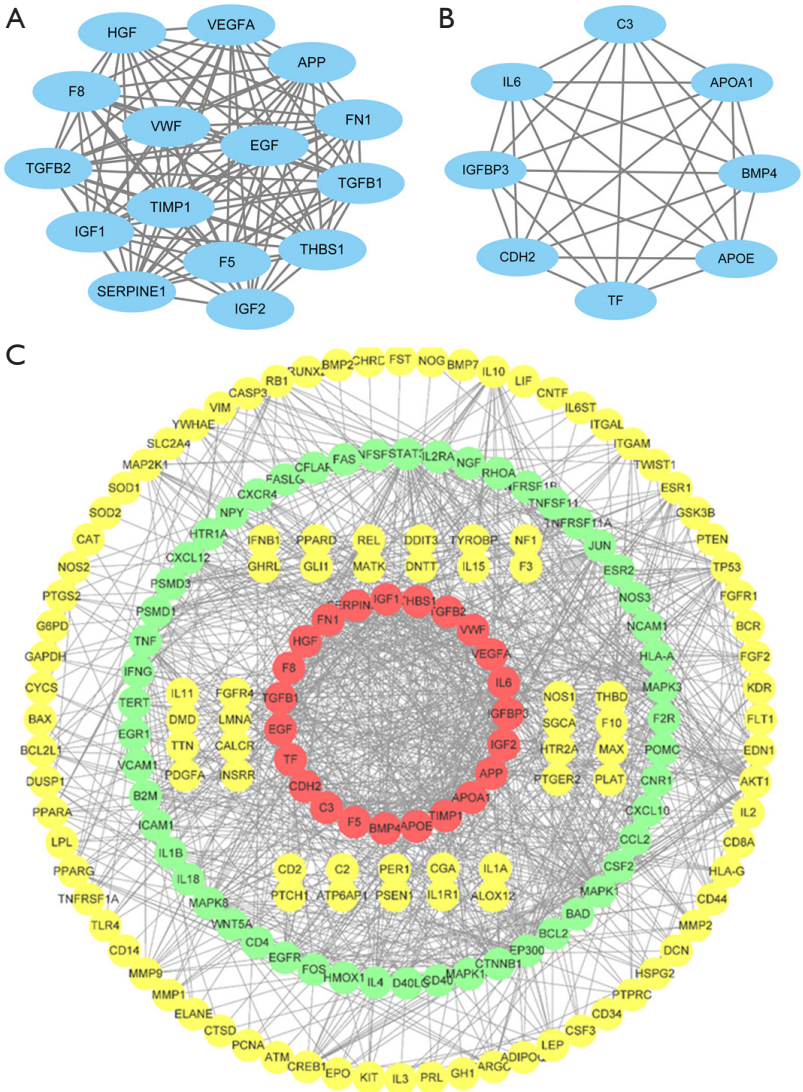


**Figure 3** PPI analysis and MCODE clusters. (A) Cluster 1: the first significant module was made up of 15 nodes and 105 edges. (B) Cluster 2: the second significant module contained 8 nodes and 28 edges. (C) The PPI network constructed by the STRING consisted of 188 genes and 1,058 edges, which was under the maximum interaction score >0.9 (high confidence). PPI, protein-protein interaction.

and Alzheimer's disease.

Being the significant part of circulatory system, *F8*, *VWF*, and *VEGFA*, may play a key role in the mechanism of SCI-induced OP. *F8* deficiency may participate in bone homeostasis by inducing acute bone loss (21), increasing bone resorption (22,23), and complexing with *VWF* via RANKL-OPG (24), resulting in OP. Some studies reported that *VEGFA* was an important target for OP treatment (25,26). Within the bone, *VEGFA* was regulated by *HIF-1* to promote bone formation, whereas decreasing the expression of *VEGFA* could inhibit bone formation thereby leading to OP (27), which is consistent with the finding of another study (28).

*HGF* was reported to be involved in the process of OP and osteoproliferation and may therefore prove to be a potential biomarker, though the pathogenic mechanism remains unclear (29). In addition, transplantation of dental pulp stem cells modified by *HGF* was found as an effective way for the prevention of early bone loss (30). *IGF1* has been identified to act on skeletal growth and may also function as one metabolic factor that results in fragility fracture (31). On the one hand, a high level of *IGF1* could

**Table 1** Potential drugs targeting genes with SCI and osteoporosis association

| Number | Drug | Gene | Type | Score* | PMID |
|---|---|---|---|---|---|
| 1 | BAN2401 | *APP* | Inhibitor | 6.17 | None |
| 2 | TB-402 | *F8* | Inhibitor | 31.90 | None |
| 3 | Drotrecogin alfa (activated) | *F8* | Inhibitor | 5.32 | None |
| 4 | Rilotumumab | *HGF* | Antibody, inhibitor | 11.39 | None |
| 5 | Ficlatuzumab | *HGF* | Antibody, inhibitor | 11.39 | None |
| 6 | Dusigitumab | *IGF1* | Inhibitor | 31.90 | None |
| 7 | Siltuximab | *IL-6* | Inhibitor | 10.21 | 8823310 |
| 8 | Olokizumab | *IL-6* | Inhibitor | 10.21 | 24641941 |
| 9 | Clazakizumab | *IL-6* | Inhibitor | 7.66 | None |
| 10 | Lerdelimumab | *TGFB2* | Inhibitor | 31.90 | None |
| 11 | Fresolimumab | *TGFB2* | Antibody, inhibitor | 10.63 | None |
| 12 | Ranibizumab | *VEGFA* | Inhibitor | 8.81 | 18046235 |
| 13 | Caplacizumab | *VWF* | Inhibitor | 13.67 | None |

Each drug-gene interaction ensured that the hypothetical drug had an expected effect on the condition, whose screening criteria was that the interacting score should be higher than 5. The link to the source was tracked to confirm the report and evaluate related metadata. Drugs that targeted the candidate genes through appropriate interactions were collected in the final list. *, the score is the combined number of database sources and PubMed references. APP, amyloid beta precursor protein; F8, coagulation factor VIII; HGF, hepatocyte growth factor; IGF1, insulin like growth factor 1; IL-6, interleukin 6; TGFB2, transforming growth factor beta 2; VWF, von Willebrand factor; VEGFA, vascular endothelial growth factor A; SCI, spinal cord injury.

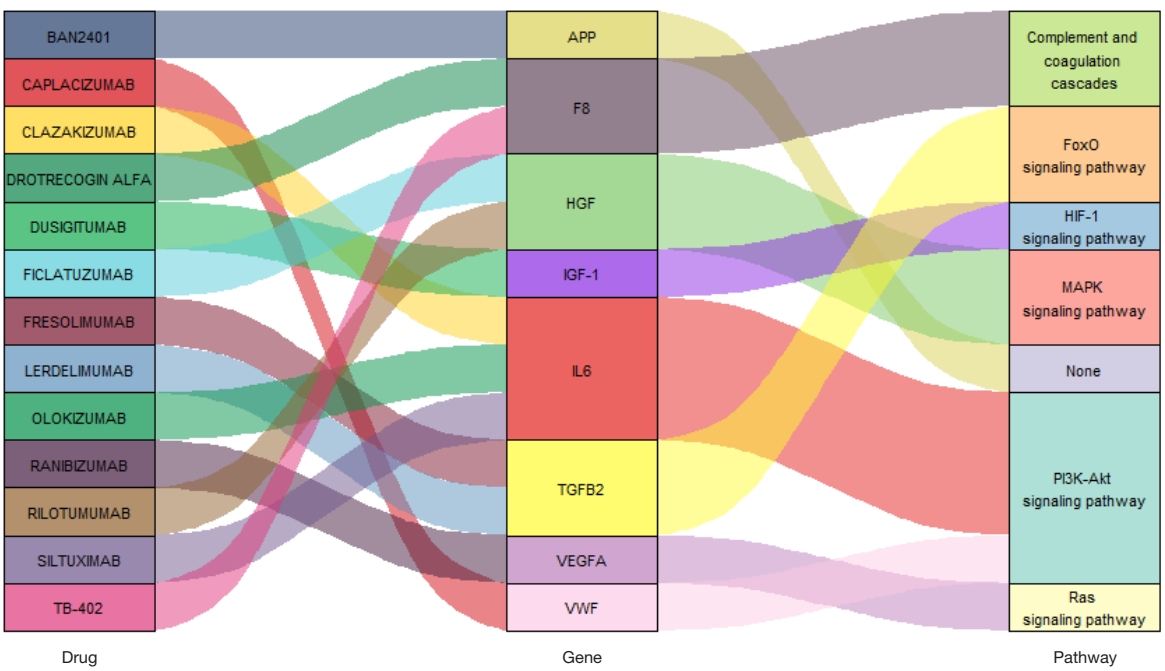


**Figure 4** Sankey diagram of drug-gene interaction. The picture displayed the drug-gene and gene-pathway interaction, containing 13 drugs targeting 8 genes and 6 pathways. APP, amyloid beta precursor protein; F8, coagulation factor VIII; HGF, hepatocyte growth factor; IGF1, insulin like growth factor 1; IL-6, interleukin 6; TGFB2, transforming growth factor beta 2; VWF, von Willebrand factor; VEGFA, vascular endothelial growth factor A; FoxO, forkhead box protein O; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-kinase; HIF-1, hypoxia-inducible factor-1.

**Table 2** Summary of BP and KEGG pathway gene set enrichment analysis

| Category | Term | Count | FDR* | Genes |
|---|---|---|---|---|
| Biological process | Platelet degranulation | 7 | 4.25E–16 | *VWF, VEGFA, TGFB2, APP, IGF1, HGF, F8* |
| Biological process | Positive regulation of peptidyl-tyrosine phosphorylation | 5 | 8.32E–11 | *VEGFA, APP, IL-6, IGF1, HGF* |
| Biological process | Positive regulation of MAPK cascade | 4 | 5.43E–08 | *VEGFA, TGFB2, IGF1, HGF* |
| Biological process | Localization of cell | 4 | 1.24E–07 | *VEGFA, APP, IL-6, IGF1* |
| Biological process | Positive chemotaxis | 3 | 1.78E–06 | *VEGFA, APP, HGF* |
| KEGG pathway | PI3K-Akt signaling pathway | 5 | 3.07E–09 | *VWF, VEGFA, IL-6, IGF1, HGF* |
| KEGG pathway | MAPK signaling pathway | 4 | 1.03E–07 | *VEGFA, TGFB2, IGF1, HGF* |
| KEGG pathway | HIF-1 signaling pathway | 3 | 7.57E–07 | *VEGFA, IL-6, IGF1* |
| KEGG pathway | FoxO signaling pathway | 3 | 1.44E–06 | *TGFB2, IL-6, IGF1* |
| KEGG pathway | Ras signaling pathway | 3 | 5.11E–06 | *VEGFA, IGF1, HGF* |

With a strict level, a P value cutoff was set. Among the most importantly enriched biological process and KEGG pathways above the cutoff, those most relevant to SCI and osteoporosis pathology were chosen from the researches and literature. *, FDR correction was performed to control for the false positive. APP, amyloid beta precursor protein; F8, coagulation factor VIII; HGF, hepatocyte growth factor; IGF1, insulin like growth factor 1; IL-6, interleukin 6; TGFB2, transforming growth factor beta 2; VWF, von Willebrand factor; VEGFA, vascular endothelial growth factor A; FoxO, forkhead box protein O; BP, biological process; FDR, fasle discovery rate; KEGG, Kyoto Encyclopedia of Genes and Genomes; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-kinase; HIF-1, hypoxia-inducible factor-1.

promote bone formation and growth rate (32). On the other hand, it is positively correlated with low bone mineral density, suggesting that a low level of *IGF1* is an indirect risk factor for fracture (33,34).

*IL-6* overexpression was found to be associated with SCI-induced OP. As an osteoclast differentiation modulator, *IL-6* can cause excessive osteoclastic activity and osteolysis by encouraging osteoclastogenesis (35). A study showed that zoledronate could further enhance osteoclast differentiation via the *IL-6*/RANKL axis (36). Interestingly, *TGFB2* was also identified as a biomarker of OP (37). The above-mentioned core genes were involved in the development of SCI as well as OP, implying that gene upregulation or downregulation after SCI may participate in the occurrence and progress of OP via various pathways such as the PI3K-Akt signaling pathway and MAPK signaling pathway, eventually causing imbalance between osteogenesis and osteoclasts and leading to OP.

Functional analysis suggested that the top five most enriched 'biological process' and KEGG pathway annotations may be the pathology of SCI-induced OP. For example, a study (38) reported that platelet degranulation participated in the occurrence and progress of OP after SCI by releasing some growth factors such as *VWF* and *VEGFA* to regulate cell proliferation, chemotaxis, and differentiation. Additionally, positive regulation of MAPK cascade and activation of Akt was reported to participate in OP by inducing bone loss (39). As mentioned previously, the PI3K-Akt signaling pathway plays a key role in the initiation and sustainment of SCI-induced OP. On the one hand, inhibition of the PI3K/Akt pathway is induced by endoplasmic reticulum stress after SCI (40), and on the other hand, PI3K/Akt signaling pathway also played a significant role in inhibiting OP by promoting osteoblast proliferation, differentiation, and bone formation (41). Therefore, based on its function, up-regulation of the PI3K/Akt signaling pathway may be a potential target for the treatment of SCI-induced OP. Experimental treatments have obtained some effective advancements in mediating PI3K/Akt signaling pathway via various methods (42). Similarly, inhibition of the MAPK signaling pathway can not only promote recovery of SCI but delay the progression of OP as well as other pathways (43-45).

As mentioned above, the expressions of *F8*, *HGF*, *IGF1*, *TGFB2*, *VWF* and *VEGFA* were downregulated while the expression of *APP* and *IL-6* was up-regulated during the progression of OP following SCI. According to the drug-gene interaction, the potential drugs siltuximab (10.21, IL-6

 

inhibitor), olokizumab (10.21, IL-6 inhibitor), clazakizumab (7.66, IL-6 inhibitor) and BAN2401 (6.17, APP inhibitor) are likely to attenuate inflammation and prevent bone loss. Siltuximab is currently studied for the treatment of COVID-19 and idiopathic multicentric Castleman disease (46-48). olokizumab is used in clinical trials for the treatment of rheumatoid arthritis with a remarkable therapeutic effect (49,50). Clazakizumab is beneficial not only for antibody-mediated rejection (51), but for active psoriatic arthritis (52). BAN2401 is mainly utilized for Alzheimer's disease due to its advantage of improving binding strength to soluble aggregates of amyloid-beta (53,54). Although these drugs have not yet been currently used in SCI-induced OP, the results of text mining and computational analysis demonstrated that hub genes are regulated after SCI involved in the OP occurrence, suggesting that drugs targetable key gene symbols have the potential to prevent the occurrence and development of SCI-induced OP.

SCI-induced OP is essentially a neurogenic bone loss process and the nerve system is found to be a necessary mediator in regulating bone cell functions, ultimately affecting bone homeostasis (55). There are three neural changes in the occurrence of the disease. Firstly, nerves are widely distributed in the bone, but the bone deprived of its innervation shows reduced bone deposition and mineralization as well as increased bone resorption (2). The other two factors are neuropeptides and denervation in SCI, which may result in a significant decrease in innervation density and neuropeptides in the bones, thus distorting the balance between bone formation and resorption.

It is common knowledge that RANKL, osteoprotegerin (OPG), sclerostin, and cathepsin K play a key role in the occurrence of OP. Experiments (56) demonstrated that the use of inhibitors of these targets could obviously delay the progression of SCI-induced OP. Osteoblasts could regulate the recruitment and activity of osteoclasts through the expression of RANKL and OPG, members of the TNF family (57). RANKL could promote osteoblast proliferation and activation via binding to its receptor RANK, while OPG acted as a receptor to bind with RANKL, thus preventing the activation of RANK. Following SCI, RANKL was upregulated by binding to the RANK receptor on osteoclastogenesis, thus leading to OP (58). These findings suggest that the RANK/RANKL/OPG axis provides a means of coupling the activities of osteoblasts and osteoclasts and controlling the balance between bone formation and resorption (59). The Wnt signaling pathway and Sclerostin also play a key role in the development of SCI-induced OP. Sclerostin is a biomarker of SCI-induced OP, playing an important role in mediating bone loss in response to unloading (60). Canonical Wnt signaling promoted bone formation by stimulating osteoblast differentiation and osteoblast growth (61). For example, several proteins involved in Wnt signaling were repressed in the distal femur and proximal tibia after SCI, while the number of osteocytes stained for Sclerostin was increased (62), which contributed to the occurrence of OP. In addition, Qin *et al.* (63) found that the Sclerostin antibody retained the structure of osteocytes and blocked the skeletal deterioration following SCI. Therefore, the proteins and signaling pathways mentioned above may be important targets for the treatment of SCI-induced OP.

Although these OP-related core genes have been validated by other laboratories, they failed to identify them as hub genes of SCI-induced OP as we did in our computational analysis. It is also extremely familiar that many potential biomarkers were obtained by text mining and bioinformatical analysis with or without verification, finally guiding us to later experimental and mining candidate medicines (64,65), which offered us a new study project that hub genes screened out by text mining combining with known genes are likely to construct novel mechanisms.

There are some limitations to this study. First, we did not perform experimental verification to enhance the credibility of this article. In addition, the criteria that we selected for screening out hub genes are subjective and the databases utilized for the bioinformatical analysis are limited; for instance, the confidence score in constructing the PPI network was determined by the researchers, and the acquisition of key genes was also closely related to the algorithm selected by the researchers. To verify the reliability of the key genes obtained by the MCODE analysis, we also used another method-cytoHubba to analyze and found that the results were completely consistent.

## Conclusions

In conclusion, the candidate drugs that target the core genes for the treatment of SCI-induced OP were investigated by text mining and computational methods. These analytic methods could be applied routinely in developing databases and analysis tools. Consequently, we obtained 13 potential drugs, including an APP inhibitor, 2 F8 inhibitors, 2 HGF

 

antibodies, an IGF1 inhibitor, 3 IL-6 inhibitors, 2 TGFB2 inhibitors, a VEGFA inhibitor, and a VWF inhibitor. Among them, siltuximab, olokizumab, clazakizumab and BAN2401 have not been tested in SCI-induced OP, which provides a curing guideline and novel targeted therapies as a potential treatment for SCI-induced OP.

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## Footnote

*Reporting Checklist:* The authors have completed the STREGA checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-21-6900/rc

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-21-6900/coif). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). We just re-analyzed the open accessed datasets (http://pubmed2ensembl.ls.manchester.ac.uk), and no ethical approval was required by the local ethics committees.



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