

結構生物資訊期末報告

一、研究動機

本學期意外在學校跌倒，導致半月板撕裂，膝蓋功能大幅衰退，需進行手術修補，也讓我認識到膝蓋周邊的組織重要性。半月板是膝關節內的纖維軟骨，負責關節與關節間的緩衝且可控制旋轉度而穩定膝關節[1]，破裂時會影響膝蓋運作，並加速膝關節退化[2]。增生療法治療(prolotherapy)如:platelet rich plasma(PRP)治療取血小板中富含修復組織所需的生長因子，提供自體修復[3]，因不須開刀而成為許多運動員的治療選項。然而，其高昂的價格使病患未必負擔得起，因此本研究將對bone morphogenetic protein(BMP2)進行分析與討論，其已被證實可作為軟骨形成因子[4]，期許能對該領域有更佳的認識，提升增生療法的使用性與廣泛性。

二、序列層級分析

使用BLAST尋找BMP2的homologous proteins，因許多物種的BMP2相似性大於90%，故選擇相似性小於95%且query cover不等於100%的gene共9個，並對其進行multiple sequence alignment(MSA)

```

ACJ350547.1 1 1 MVAGT--HCLLALLLQDLLGRAGLLPELGRKKFAASTGRS--SSQPSDDVLSEFELRLLSMFGLKQRPTPSRDGVVPP 76
ACJ350551.1 1 1 MVAGT--RCLLALLLPQVLGGAGLLPELGRKKFAASTGLS--SSQPSDDVLSEFELRLLSMFGLKQRPTPSRDGVVPP 76
XP_060140626.1 1 1 MVAGT--RCLLALLLPQVLGGVAGLLPELGRKKFAVSAGRS--SSQPSDDVLSEFELRLLSMFGLKQRPTPSRDGVVPP 76
XP_059887203.1 1 1 MVAGT--RCLLALLLPQVLGGVAGLLPELGRKKFAVSAGRS--SSQPSDDVLSEFELRLLSMFGLKQRPTPSRDGVVPP 76
XP_024620013.1 1 1 MVAGT--RCLLALLLPQVLGGVAGLLPELGRKKFAVSAGRS--SSQPSDDVLSEFELRLLSMFGLKQRPTPSRDGVVPP 76
XP_067563606.1 1 1 MVAGT--RCLLALLLPQVLGGVAGLLPELGRKKFAVSAGRS--SSQPSDDVLSEFELRLLSMFGLKQRPNPSRDGVVPP 76
XB056452.1 1 1 MCPRTpeRRSRRGFNMLALNGTICGLLK-----VDHGGRRDp1SSSPSDEVLSSEFELRLLSMFGLKQRPTPSRDGVVPP 73
XP_045013476.1 1 1 MVAGT--RCLLALLLPQVLGGVAGLLPELGRKKFAVSAGRS--SSQPSDDVLSEFELRLLSMFGLKQRPTPSRDGVVPP 76
KAI5277791.1 1 1 MQITPrr5SGAGDEPAEVLGSAIIFPELGQRRAASAGSS--SSQPSDDVLSEFELRLLSVFGPKRRPTPIRVAVPP 78

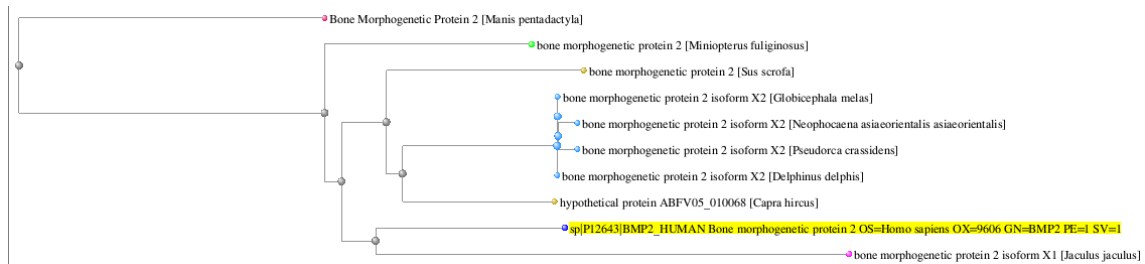
ACJ350547.1 77 YMLDLYRRHSQQGAPADPHRLERAASLANTVRSFHHEESLEELPEMSGKTTTRFFNLTSPITEEFITSaelQVfREQ 156
ACJ350551.1 77 YMLDLYRRHSQQGAPADPHRLERAASLANTVRSFHHEESLEELPEMSGKTTTRFFNLTSPITEEFITSaelQVfREQ 156
XP_060140626.1 77 YMLDLYRRHSQQGAPADPHRLERAASLANTVRSFHHEESLEELPEISGKTTTRFFNLTSPITEEFITSaelQVfREQ 156
XP_059887203.1 77 YMLDLYRRHSQQGAPADPHRLERAASLANTVRSFHHEESLEELPEISGKTTTRFFNLTSPITEEFITSaelQVfREQ 156
XP_024620013.1 77 YMLDLYRRHSQQGAPADPHRLERAASLANTVRSFHHEESLEELPEISGKTTTRFFNLTSPITEEFITSaelQVfREQ 156
XP_067563606.1 77 YMLDLYRRHSQQGAPADPHRLERAASLANTVRSFHHEESLEELPEISGKTTTRFFNLTSPITEEFITSaelQVfREQ 156
XB056452.1 74 YMLDLYRRHSQQGAPADPHRLERAASLANTVRSFHHEESLEELPEMSGKTTTRFFNLTSPITEEFITSaelQVfREQ 153
XP_045013476.1 77 YMLDLYRRHSQQGAPADPHRLERAASLANTVRSFHHEESLEELPEVNGRSTRFFNLTSPITEEFITSaelQVfREQ 156
KAI5277791.1 79 YTLDL--RRHSQQGAPVPDRRLERAASLADTVRSFHHEESLEELPETSgKTTTRFFNLTSPITEEFITSaelQVfREQ 157

ACJ350547.1 157 QETLENNSSFHHRINIYEIKPAASHSQLPVTRLDDLRLVQNASRMESFDVTPAVMRITAGQLANHGFVVEVAHPEDSH 236
ACJ350551.1 157 QETLENNSSFHHRINIYEIKPATANSKFPVTRLDDLRLVTPHNASRMESFDVTPAVMRITAGQLANHGFVVEVAHPEDSH 236
XP_060140626.1 157 QETLENNSSFHHRINIYEIKPATANSKFPVTRLDDLRLVTPHNASRMESFDVTPAVMRITAGQLANHGFVVEVAHPEDSH 236
XP_059887203.1 157 QETLENNSSFHHRINIYEIKPATANSKFPVTRLDDLRLVTPHNASRMESFDVTPAVMRITAGQLANHGFVVEVAHPEDSH 236
XP_024620013.1 157 QETLENNSSFHHRINIYEIKPATANSKFPVTRLDDLRLVTPHNASRMESFDVTPAVMRITAGQLANHGFVVEVAHPEDSH 236
XP_067563606.1 157 QETLENNSSFHHRINIYEIKPATANSKFPVTRLDDLRLVTPHNASRMESFDVTPAVMRITAGQLANHGFVVEVAHPEDSH 236
XB056452.1 154 PEALENNSSFHHRINIYEIKPATANSKFPVTRLDDLRLVTPHNASRMESFDVTPAVMRITAGQLANHGFVVEVAHPEDSH 233
XP_045013476.1 157 QETLENNSSFHHRINIYEIKPEAASSTFPVTRLDDLRLVTPHNASRMESFDVTPAVMRITAGHTNHGFVVEVZHLQEK 236
KAI5277791.1 158 QGTLENNSSFHHRINIYEIKPATANSKFPVTRLDDLRLVTPHNASRMESFDVTPAVMRITAGQLANHGFVVEVAHLAENQ 237

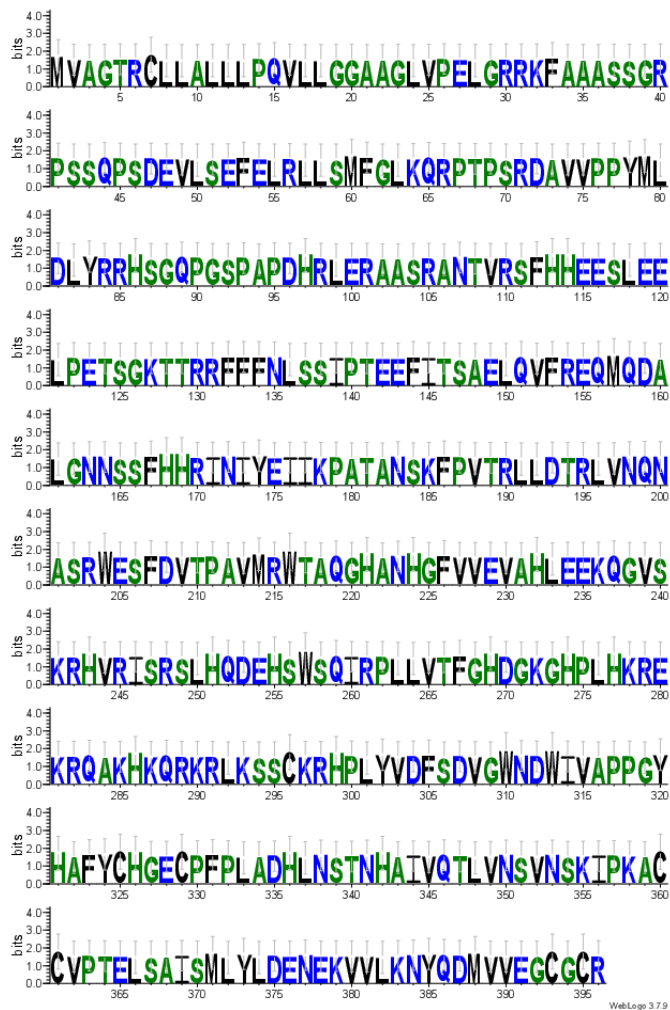
ACJ350547.1 237 GVSKRHRVIRSLHQDEHSQSQRPLLVTFGHDGKGHPLHREKRQAKHKQRRLKSSCKRHPLVYDFSDVGNDWIVAP 316
ACJ350551.1 237 EVSKRHRVIRSLHQDEHSQSQRPLLVTFGHDGKGHPLHREKRQAKHKQRRLKSSCKRHPLVYDFSDVGNDWIVAP 316
XP_060140626.1 237 GVSKRHRVIRSLHQDKHSQSQRPLLVTFGHDGKGHPLHREKRQAKHKQRRLKSSCKRHPLVYDFSDVGNDWIVAP 316
XP_059887203.1 237 GVSKRHRVIRSLHQDKHSQSQRPLLVTFGHDGKGHPLHREKRQAKHKQRRLKSSCKRHPLVYDFSDVGNDWIVAP 316
XP_024620013.1 237 GVSKRHRVIRSLHQDKHSQSQRPLLVTFGHDGKGHPLHREKRQAKHKQRRLKSSCKRHPLVYDFSDVGNDWIVAP 316
XP_067563606.1 237 GASKRHRVIRSLHQDKHSQSQRPLLVTFGHDGKGHPLHREKRQAKHKQRRLKSSCKRHPLVYDFSDVGNDWIVAP 316
XB056452.1 234 GASKRHRVIRSLHQDEHSQSQRPLLVTFGHDGKGHPLHREKRQAKHKQRRLKSSCKRHPLVYDFSDVGNDWIVAP 313
XP_045013476.1 237 GVSKRHRVIRSLHQDEHSQSQRPLLVTFGHDGKGHPLHREKRQAKHKQRRLKSSCKRHPLVYDFSDVGNDWIVAP 316
KAI5277791.1 238 GVSKRHRVIRSLHQDEHSQSQRPLLVTFGHDGKGHPLHREKRQAKHKQRRLKSSCKRHPLVYDFSDVGNDWIVAP 317

ACJ350547.1 317 PGYHAFYCHGECPPFLADHLNSTHAIQVTLVNSVNSKIPKACCVPTLSAISHLYLENEK VV1knYQ0MVVEG- 391
ACJ350551.1 317 PGYHAFYCHGECPPFLADHLNSTHAIQVTLVNSVNSKIPKACCVPTLSAISHLYLENEK VV1knYQ0MVVEG- 391
XP_060140626.1 317 PGYHAFYCHGECPPFLADHLNSTHAIQVTLVNSVNSKIPKACCVPTLSAISHLYLENEK V-----STLWTFES 387
XP_059887203.1 317 PGYHAFYCHGECPPFLADHLNSTHAIQVTLVNSVNSKIPKACCVPTLSAISHLYLENEK V-----STLWTFES 387
XP_024620013.1 317 PGYHAFYCHGECPPFLADHLNSTHAIQVTLVNSVNSKIPKACCVPTLSAISHLYLENEK V-----STFWTFES 387
XP_067563606.1 317 PGYHAFYCHGECPPFLADHLNSTHAIQVTLVNSVNSKIPKACCVPTLSAISHLYLENEK V-----STLWTFES 387
XB056452.1 314 PGYHAFYCHGECPPFLADHLNSTHAIQVTLVNSVNSKIPKACCVPTLSAISHLYLENEK VV1knYQ0MVVEG 389
XP_045013476.1 317 PGYHAFYCHGECPPFLADHLNSTHAIQVTLVNSVNSKIPKACCVPTLSAISHLYLENEK [11]VC---FTQAGFPPT 400
KAI5277791.1 318 PGYHAFYCHGECPPFLADHLNSTHAIQVTLVNSVNSKIPKACCVPTLSAISHLYLENEK VV1knYQ0MVVEG 393
```

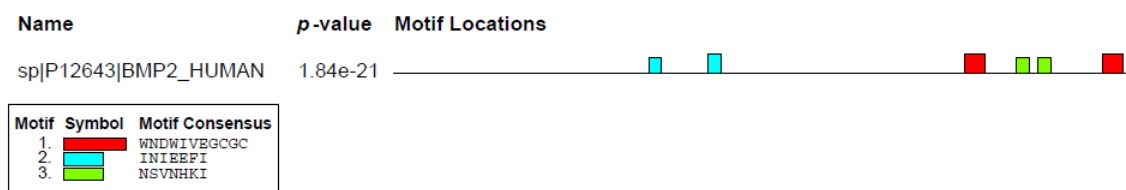
distance tree:



weblogo 3:視覺化呈現序列保守性，協助識別motif



memes suite:找尋序列中已知的motif

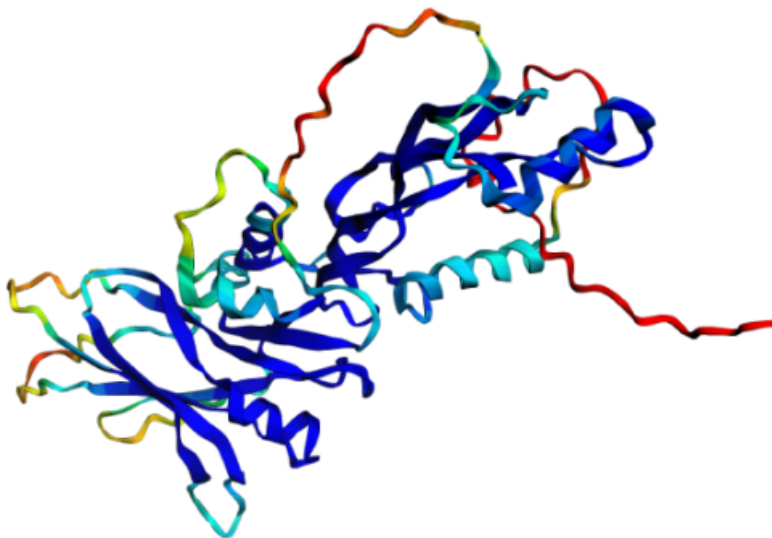


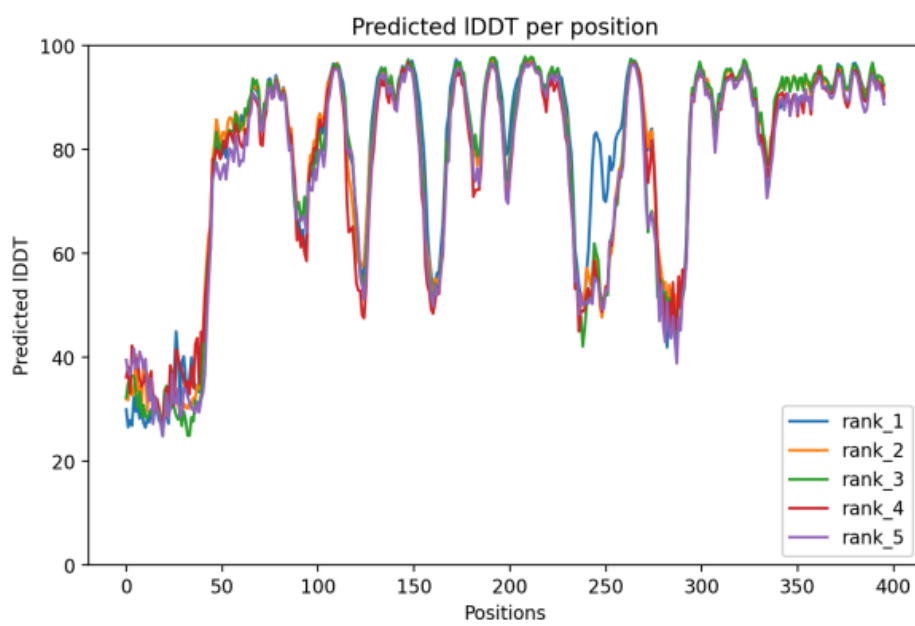
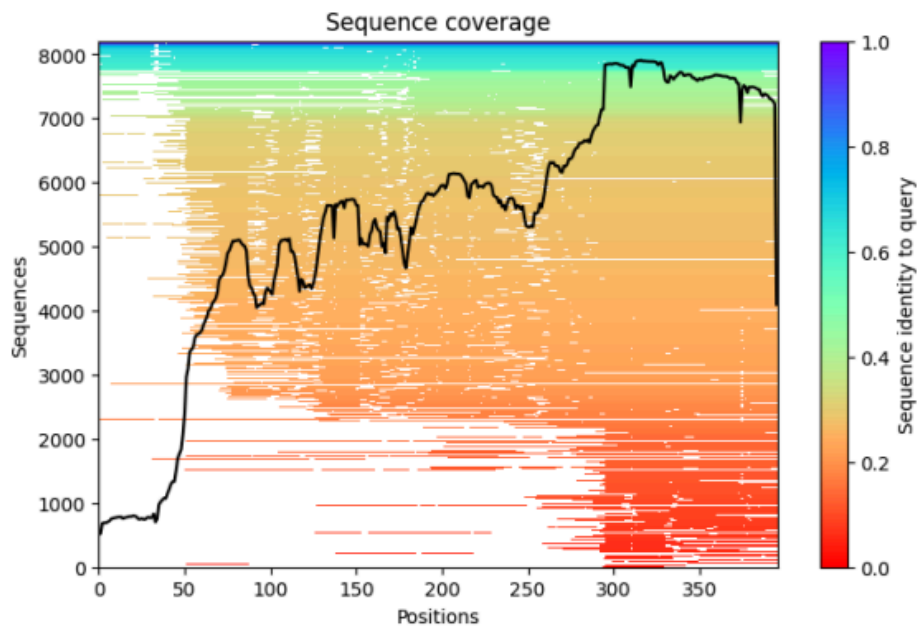
amino acid composition(AAC)分析

	Name ?	Freq. ?
A	Alanine	0.0707
C	Cysteine	0.0202
D	Aspartic acid	0.0379
E	Glutamic acid	0.0657
F	Phenylalanine	0.0429
G	Glycine	0.0556
H	Histidine	0.053
I	Isoleucine	0.0303
K	Lysine	0.048
L	Leucine	0.0985
M	Methionine	0.0177
N	Asparagine	0.0404
P	Proline	0.0631
Q	Glutamine	0.0404
R	Arginine	0.0783
S	Serine	0.0884
T	Threonine	0.0429
V	Valine	0.0732
W	Tryptophan	0.0126
Y	Tyrosine	0.0202

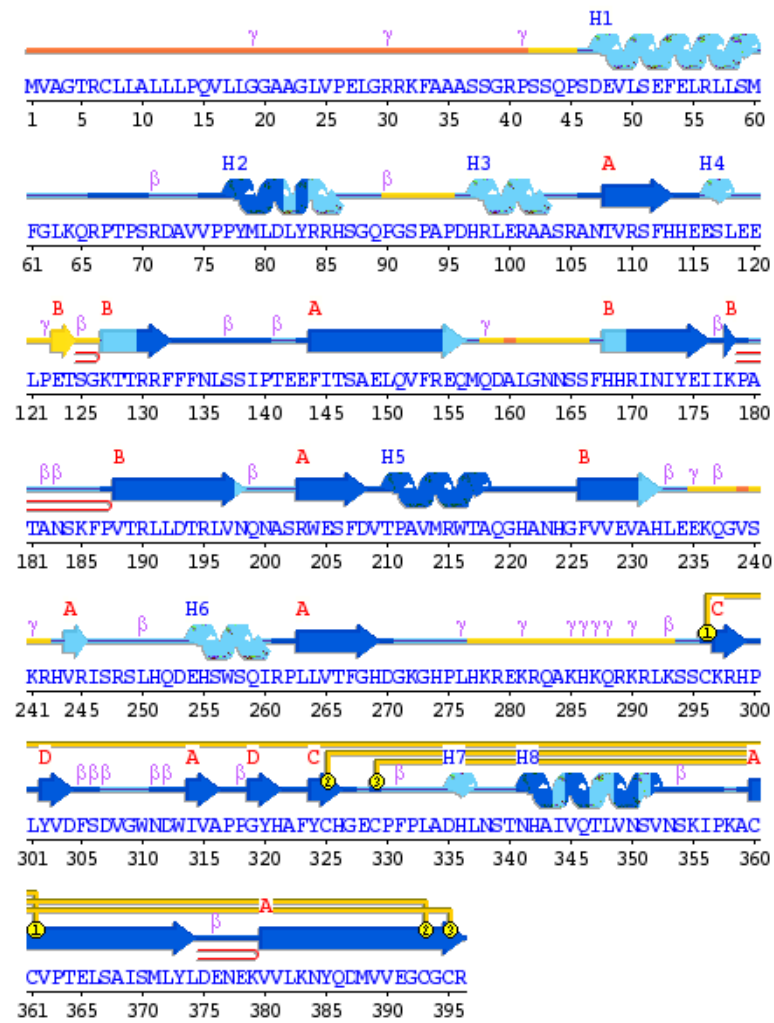
三、結構層級分析

使用alpha fold 2進行結構預測，並獲取分析結果



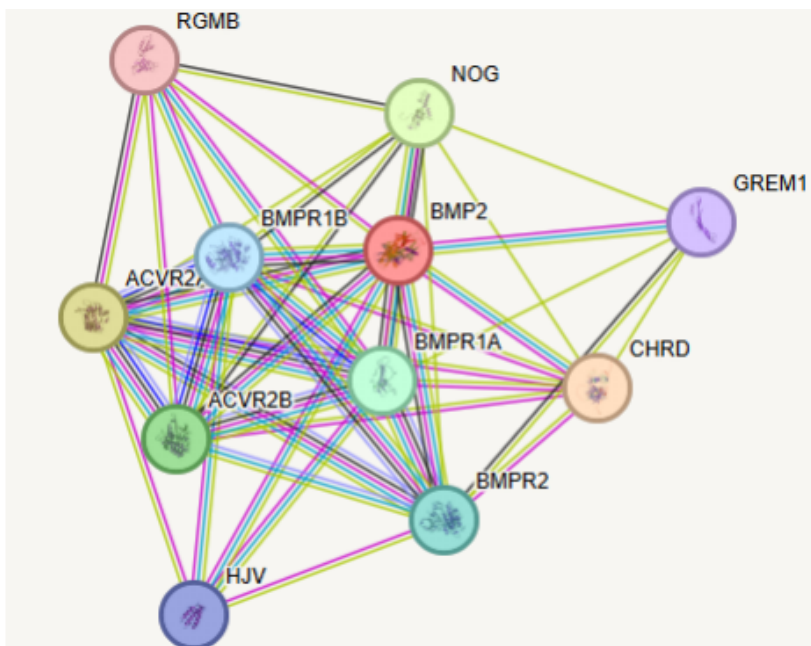


PDBsum:進行結構分析



四、pathway分析

STRING:protein-protein interaction的數據庫



Your Input:

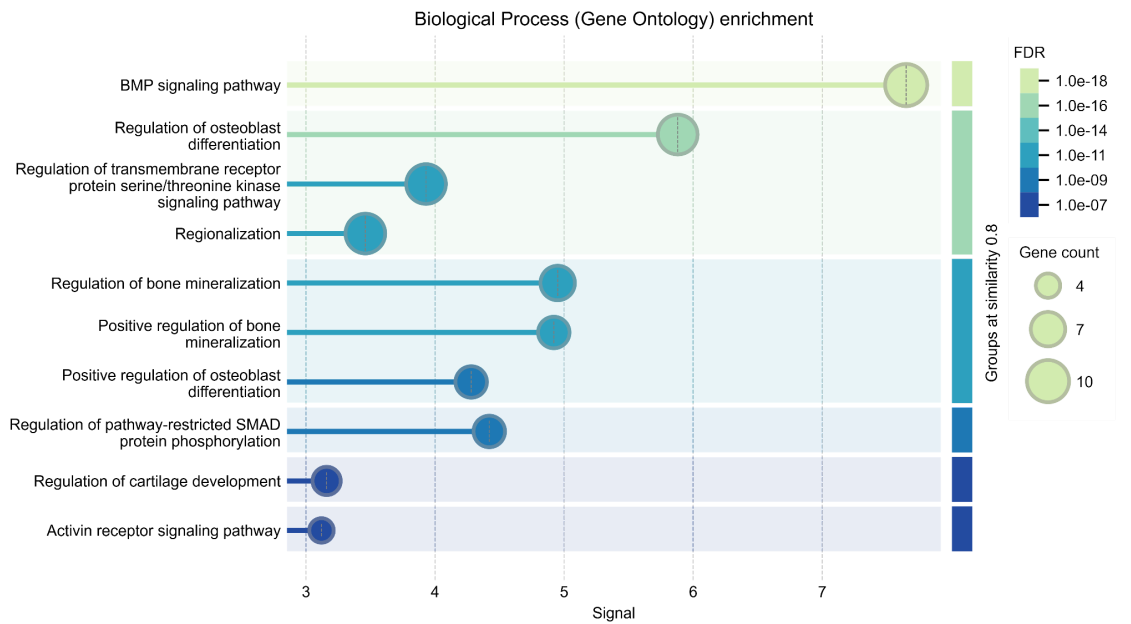
BMP2

Bone morphogenetic protein 2; Induces cartilage and bone formation. Stimulates the differentiation of myoblasts into osteoblasts via the EIF2AK3-EIF2A- ATF4 pathway. BMP2 activation of EIF2AK3 stimulates phosphorylation of EIF2A which leads to increased expression of ATF4 which plays a central role in osteoblast differentiation. In addition stimulates TMEM119, which upregulates the expression of ATF4; Belongs to the TGF-beta family. (396 aa)

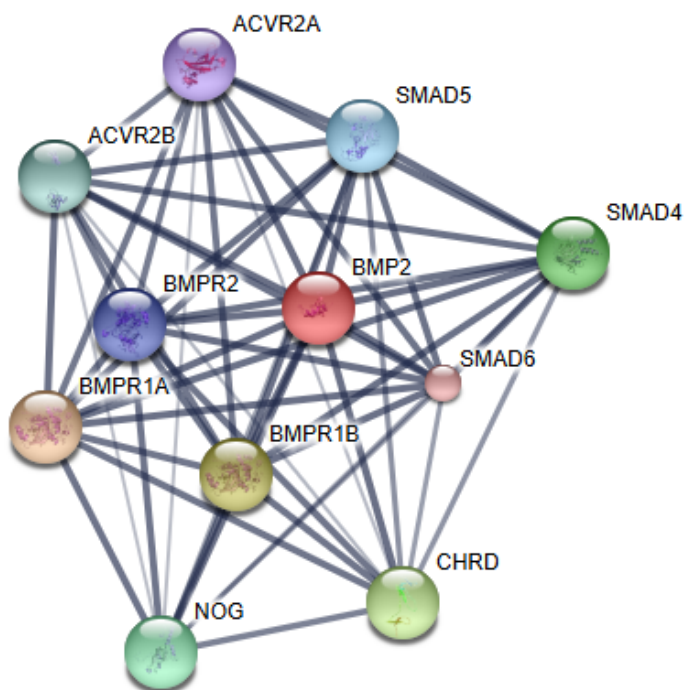
Predicted Functional Partners:

		Neighborhood	Gene Fusion	Cooccurrence	Coexpression	Experiments	Databases	Textmining	[Homology]	Score
CHRD	Chordin; Dorsalizing factor. Key developmental protein that dorsalizes early vertebrate embryonic tissues by binding to ventral...									0.999
ACVR2A	Activin receptor type-2A; On ligand binding, forms a receptor complex consisting of two type II and two type I transmembrane ...									0.999
NOG	Noggin; Inhibitor of bone morphogenetic proteins (BMP) signaling which is required for growth and patterning of the neural tu...									0.999
ACVR2B	Activin receptor type-2B; Transmembrane serine/threonine kinase activin type-2 receptor forming an activin receptor complex ...									0.999
BMPR1A	Bone morphogenetic protein receptor type-1A; On ligand binding, forms a receptor complex consisting of two type II and two ty...									0.999
BMPR2	Bone morphogenetic protein receptor type-2; On ligand binding, forms a receptor complex consisting of two type II and two ty...									0.999
BMPR1B	Bone morphogenetic protein receptor type-1B; On ligand binding, forms a receptor complex consisting of two type II and two ty...									0.999
HJV	Hemojuvelin; Acts as a bone morphogenetic protein (BMP) coreceptor. Through enhancement of BMP signaling regulates hep...									0.996
GREM1	Gremlin-1; Cytokine that may play an important role during carcinogenesis and metanephric kidney organogenesis, as a BMP ...									0.995
RGMB	RGM domain family member B; Member of the repulsive guidance molecule (RGM) family that contributes to the patterning of...									0.993

KEGG Pathways					
pathway	description	count in network	strength	signal	false discovery rate
hsa04350	TGF-beta signaling pathway	11 of 91	2.34	9.48	1.37e-23
hsa05418	Fluid shear stress and atherosclerosis	5 of 129	1.84	2.67	6.75e-07
hsa04550	Signaling pathways regulating pluripotency of stem cells	5 of 141	1.8	2.59	7.80e-07
hsa04060	Cytokine-cytokine receptor interaction	6 of 282	1.58	2.25	6.74e-07
hsa04390	Hippo signaling pathway	4 of 154	1.67	1.77	8.44e-05



STITCH:chemicals-protein interaction的數據庫



KEGG:涵蓋細胞層級網路、酵素反應、genome等資料庫

Pathway	map04060	Cytokine-cytokine receptor interaction
	map04350	TGF-beta signaling pathway
	map04390	Hippo signaling pathway
	map05200	Pathways in cancer
	map05217	Basal cell carcinoma
Disease	H00482	Brachydactyly
	H02481	Syndromic disorder with short stature

五、virtual screening

六、統整與結論

1. 序列層級分析

BLAST和Motif分析可知BMP2帶有TGF-beta domain且在不同物種中高度保守，代表了其對骨骼與軟骨發育的重要性，並作為調控因子在進化中扮演關鍵的角色。

2. 結構層級分析

了解protein結構以及特定氨基酸殘基的位置，能在基因工程或藥物設計時，了解受體或ligand的特定結合區域，增加研發效率。

3. pathway分析

BMP2與其他骨與軟骨生成的相關蛋白如:SMAD、ACVR彼此具有高可信度的交互作用，提供治療的方向。而TGF-beta也是其中的關鍵，在骨生成、組織修復具重要作用，可能促進軟骨及基質生成。

綜上所述, BMP2是一種高度保守的多功能蛋白, 其序列與結構特徵支持了它在骨骼和軟骨修復中的核心作用。通過 pathway分析可以看出, BMP2通過調控 TGF-beta訊號傳遞中的關鍵節點, 參與了組織修復及再生過程。這些研究結果表明, BMP2在半月板修復中具有潛力, 可作為未來治療策略的新方向, 並進一步探索其與其他protein或化合物的交互作用。

七、心得

本次報告最令我有感的是, 在分析生物資訊的數據時, 時常會有許多可能的結果, 如何選擇有用的資料並進行分析是件不容易的事。此外, 有關人體的調控非常複雜, protein間的關係環環相扣, 該如何尋找切入點也是門學問。也期許未來能出現有效治療膝關節相關疾病的治療方法, 造福更多患者。

八、資料來源

[1]<https://www.rah.com.tw/page/news/show.aspx?num=3366&kind=35&page=1&lang=TW>

[2]<https://kb.commonhealth.com.tw/library/52.html#data-9-collapse>

[3]<https://www.vghtc.gov.tw/UnitPage/RowViewDetail?WebRowsID=04766cfc-0731-4b75-8893-da969aa73518&UnitID=ed2fa610-1b99-4a70-b977-918c87e28e25&CompanyID=e8e0488e-54a0-44bf-b10c-d029c423f6e7&UnitDefaultTemplate=1>

[4]Claus, S., Aubert-Foucher, E., Demoor, M., Camuzeaux, B., Paumier, A., Piperno, M., ... & Mallein-Gerin, F. (2010). Chronic exposure of bone morphogenetic protein-2 favors chondrogenic expression in human articular chondrocytes amplified in monolayer cultures. *Journal of cellular biochemistry*, 111(6), 1642-1651.