Material and methods

A network that includes genes related to bone regeneration was construct and analyze to study the hub genes of a network for recognizing potentially the most important genes in the network. the bone regeneration related genes were collected from the regenerative gene database(http://regene.bioinfo-minzhao.org/). Based on regeneration gene databases 45 different gene is collaborating in bone regeneration process and here you can see the all gnes from this database(table1)

In this research we use protein-protein interaction(PPI) data from STRING (https://string-db.org/) .the PPI network was constructed by python package networkx and Cytoscape .centrality analysis is applied with networkx python package .centrality measurements were applied on network analysis to figure out the hub genes . 3 different types of centrality used: degree centrality, closeness centrality, and betweenness centrality. we use three different functions of the networkx library for calculating centrality :1. degree_centrality 2. closeness_centrality 3. betweenness_centrality .degree centrality for node x is a fraction of nodes that are connected to node x(equation1).Closeness centrality of a node x is the reciprocal of the average shortest path distance to x overall n-1 reachable nodes(n is the total number of nodes)(equation2).betweenness centrality of a node x is the sum of fraction all-pairs shortest paths through x.(equation3)

$$C(u) = \frac{n-1}{\sum_{v=1}^{n-1} d(v,u)} \quad (equation1)$$

$$c_B(v) = \sum_{s,t \in V} \frac{\sigma(s,t|v)}{\sigma(s,t)} (equation2)$$
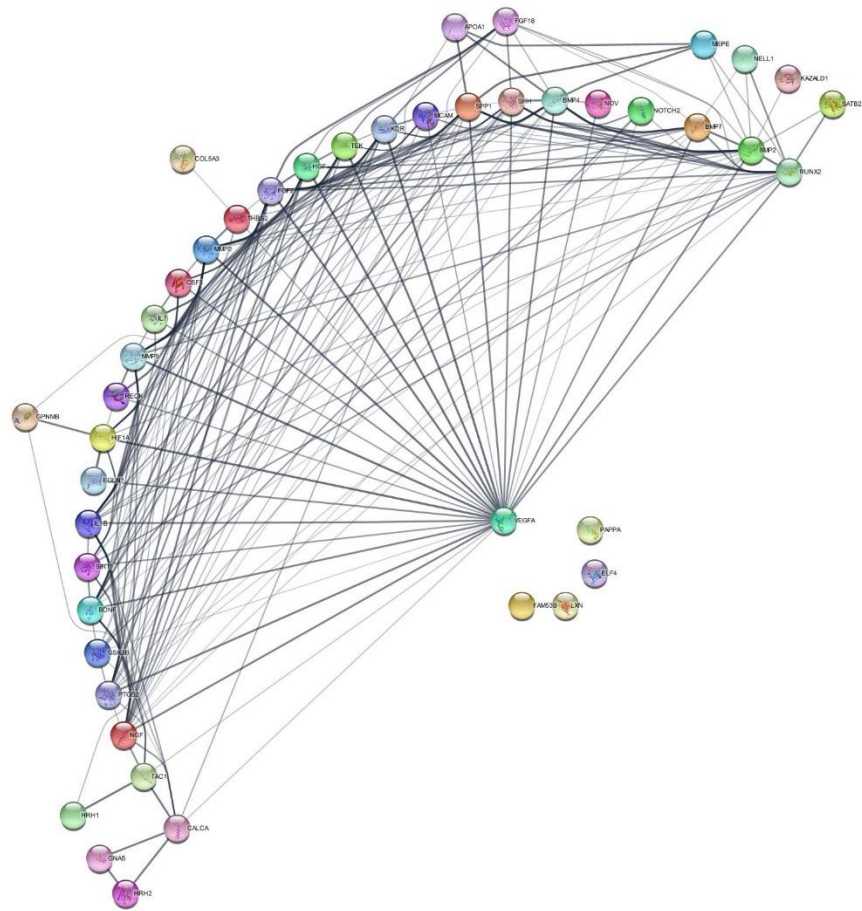
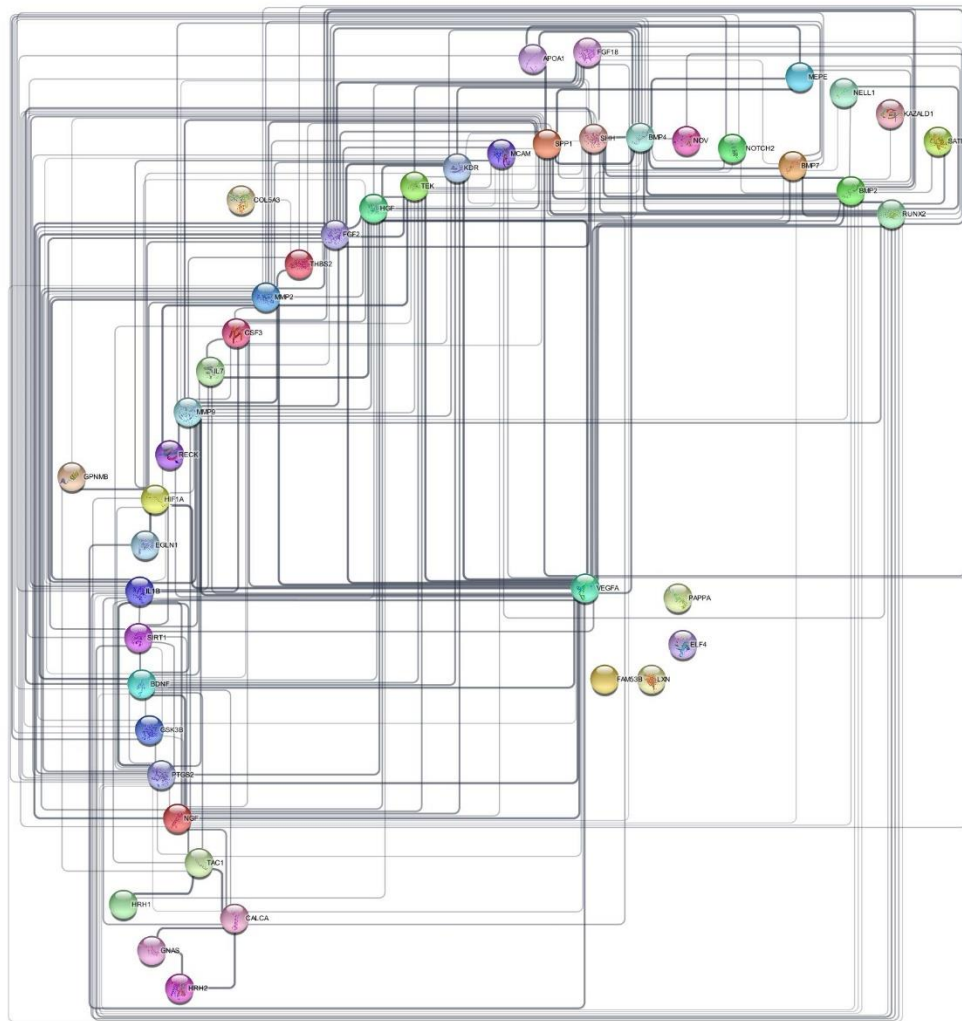*Figure 1 A protein protein interaction network constructed by STRING database*

Figure 2 A protein protein interaction network constructed by STRING database

Table 1 the list of proteins that is participated in bone regeneration.

no	name
1	VEGFA
2	FGF2
3	SPP1
4	BMP2
5	HGF
6	NGF
7	MMP9
8	BMP4
9	IL1B
10	MMP2
11	PTGS2
12	KDR
13	RUNX2
14	BDNF
15	SHH
16	TEK
17	HIF1A
18	CSF3
19	BMP7
20	MCAM
21	SIRT1
22	FGF18
23	CALCA
24	GSK3B
25	TAC1
26	THBS2
27	IL7
28	NOTCH2
29	MEPE
30	RECK
31	APOA1
32	NELL1
33	GPNMB
34	HRH2
35	SATB2
36	GNAS
37	EGLN1
38	HRH1
39	NOV
40	COL5A3
41	KAZALD1
42	PAPPA
43	FAM53B
44	ELF4
45	LXN

Result

The network for bone regeneration is shown based on centrality(fig1,2) The centrality measures of nodes in bone regeneration PPI are calculated and the top 10 genes based on three centrality measurements is identified and merged together then we have 12 hub genes based on the centrality measurements

(fig3a,b,c) that sorted by betweenness values are shown in table 1.top 5 genes based on centrality analysis is the same that indicate the high cohesence of three centrality measurments.

Table 2 12 hub genes sorted based on betweenness

Gene	Betweenness centrality	Degree centrality	Closeness centrality
VEGFA	0.21102131	0.568965517	0.674418605
Bmp4	0.12778318	0.379310345	0.591836735
FGF2	0.114579513	0.413793103	0.597938144
Bmp2	0.105782103	0.396551724	0.591836735
Spp1	0.080428611	0.413793103	0.604166667
BDNF	0.068600505	0.327586207	0.585858586
CALCA	0.051083661	0.155172414	0.483333333
RUNX2	0.043619846	0.327586207	0.552380952
PTGS2	0.035379314	0.362068966	0.568627451
MMP9	0.035329043	0.379310345	0.552380952
HIF1A	0.026679642	0.25862069	0.513274336
Bmp7	0.019011324	0.224137931	0.527272727

Distributions of closeness and degree centrality show a high correlation between theses two centrality measurements (fig3a), distribution between closeness centrality and betweenness centrality figure out that some proteins have low betweenness and relatively high closeness(fig3b)finally in a distribution of betweenness and degree we can recognize some nodes that they have low centrality degree but higher betweenness.
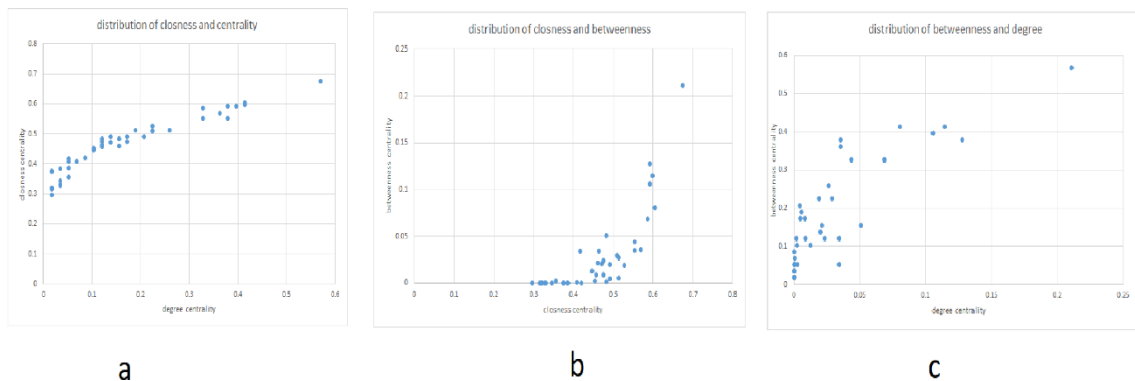

Figure 3 distribution of different centrality measurments,a.degreee-closness,b.betweenness -closness,c.betweenness-degree