ORIGINAL ARTICLE

Arvand Journal of Health & Medical Sciences

Evaluation of protein clustering of pancreatic cancer

Akram Safaei[1], Mostafa Rezaei Tavirani[2], Afsaneh Arefi Oskouei[3], Mona Zamanian Azodi[2], Seyed Reza Mohebbi[4]

[1]Students' Research Committee, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[2]Proteomic research center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[3]Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[4]Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

✉ **Reprint or Correspondence**: Mostafa Rezaei Tavirani; PhD Proteomic research center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
@✉ Tavirany@yahoo.com.

ABSTRACT

Pancreatic cancer (PC) is one of the most malignant tumors with a very poor prognosis. Late diagnosis and lack of effective therapeutic method is the weak point in pancreatic cancer therapy. For pancreatic cancer clustering analysis, thirty proteins were selected from Wiki pathway and sequence comparison was prepared by Needleman-Wunsch algorithm. Similarity scores were obtained by comparing of these proteins and the relevant dissimilarity matrix was constructed. Graphical illustrations of clustering data were performed by R software using package cluster to find subgraphs with maximal density. Finally the interactions of these proteins with their neighbors that was obtained from Mentha, Reactome-Fls Databases by the application of PSICQUIC source in Cytoscape software are presented. The result of this study showed that CREB-binding protein and epidermal growth factor receptor are reported in Wiki Pathway Androgen receptor and most significant hub that involved in pancreatic cancer, but not in the high close relationship. In conclusion cell cycle and signaling pathway have highlighted its importance in pancreatic cancer, as well as its potential as a therapeutic target in PC.

Keywords: Clustering, Pancreatic cancer, Protein-protein interaction, Cytoscape, Quick GO.

***Received*: 13 March 2016 *Accepted*: 19 May 2016**

Introduction

Pancreatic cancer (PC) is one of the most mortal caners among human. Infrequent, it takes up 3 % of all reported cases of cancer it also eighth most common cause of cancer-related deaths in worldwide (1-3). For researches it's important to find effective methods to pancreatic cancer treatment because most of patients die in the first 12 months and only 4 percent survive 5 years after diagnosis (4). Late diagnosis and lack of effective therapeutic for pancreatic cancer are related to aggressive biological phenotype of this disease from the beginning of the formation and development and rapid metastatic extension and is relatively resistant to radiation therapy and/or

Please cite as: **Safaei A, Rezaei Tavirani M, Arefi Oskouei A, Zamanian Azodi M, Mohebbi SR. Evaluation of protein clustering of pancreatic cancer. Arvand J Health Med Sci 2016;1(2):68-77.**

chemotherapy (5). To improve the treatment for patients with pancreatic cancer, the diagnosis should be made at an early stage which it requires to specific and sensitive biological markers (6). Accordingly it's an urgent requirement to promote knowledge from molecular basic of pancreatic cancer to find appropriate treatment (7, 8). Recently, researches focus on molecular alteration in pancreatic cancer (9). It has been suggested the development of pancreatic cancer need special mutations in multi-step processes (10). Chromosomal instability, polyploidy and aneuploidy are common features of many tumors including pancreatic cancer (11).

Several pancreatic cancer genes have been established, including both low-penetrance genes such as the *ABO* blood group locus (12) and high-penetrance genes such as PALB2 (13), BRCA2 (14) STK11(15) and p16/CDKN2 (16). Also, some protein alterations in pancreatic cancer has been reported in tissue (S100A11, galectin 3) (17), serum and plasma (APOC1, APOC2(18), PERK1/2, Pc-AMP, HSP27) (19) and in urine (annexin A2, gelsolin, CD59) (20). The importance of human molecular interaction networks not only summarization in reveals protein function interaction between two or more proteins but also it clear view of fundamental human biology as well as disease progression, diagnosis (21). Therefore, it is crucial to establish high sensitive algorithms to evaluate data to the better understanding of functional association of the disease protein (22). Data mining is the computational process of discovering patterns in large data sets which its goal is to make understandable structure of extracted information from a data set for further use (23). Clustering is the task of grouping a set of similar objects in the same group so that the grouped objects are more similar than to those in the others. In fact, It is the main task of discovering data mining (24, 25). This algorithm plays an essential role in the biological networks analysis, and it can be used to uncover functional modules and acquire concepts about the cellular organization (26). In previous study, it has been shown PPI network analysis confirm that the proteins with similarity in sequence and onthology are also in the close relationship (27).

The aim of present study was to evaluate the relationship between similarity in sequence and biological process via cluster analysis and also the assessment of their interactions to obtain a better understanding of the molecular basis of the pancreatic cancer to find sensitive points to introduce possible biomarker(s) and drug target discovering.

Materials and Methods

Proteins and their relationship to pancreatic cancer were identified and analyzed. Proteins were selected from Wiki pathway and then their human UniProt codes were retrieved for extracting protein sequences from UniProt database and then, sequence comparison was done by Needleman-Wunsch algorithm (pair-wise alignment) between each set of two proteins available in EBI Database. Similarity scores were obtained by comparing of these 30 proteins and the relevant dissimilarity matrix was constructed that used for clustering analysis. clustering can be useful for understanding the annotation of protein molecules and their relationship to many diseases (28). Agglomerative hierarchical clustering analysis (bottom - up) was conducted.

Graphical illustrations of clusters of data were performed by R software using package cluster to find subgraphs with maximal density. R software is a free statistical software available on (www.r-project.org). In this study silhouette plots and

dendrogram are the graphical representations. The data was analyzed by correlation distance mode. UPGMA (Unweighted Pair Group Method using arithmetic Averages) is the method that used to define the distance between clusters as the average distance (29). It is probably the most popular algorithm for hierarchical data clustering, especially in computational biology (30).

Furthermore, the annotation terms based on (GO) features was obtained using Quik GO (https://www.ebi.ac.uk/QuickGO) (31). In this study sequence closed protein for hubs collected in table 4 and their biological process was brought from Quik GO and the origin pathway surveyed in Reactome database (http://www.reactome.org/PathwayBrowser). Cytoscape v:3.2.1 also was used for the visualization of predicted interactions of the proteins (32).

Results

Clustering, as an appropriate statistical procedure, is the essential task in automatic processing that used to natural groupings of data items. Hierarchical clustering methods aim to categorize data items into a hierarchical set of clusters organized in a tree structure (30). UPGMA is the most popular hierarchical clustering algorithm in use for protein sequence clustering (33) and gene expression (34).

Enrichment analysis of the interest proteins can reveal the mechanism of the disease pathology by classification of gene/proteins according the roles in cell and discovering the shared functions between genes/proteins (35, 36). Among enrichment analysis tools the Gene Ontology (GO) is the one of the most common use to underlying functions (37). Related proteins to pancreatic cancer that extracted from Wiki Pathway database has been shown in Table 1.

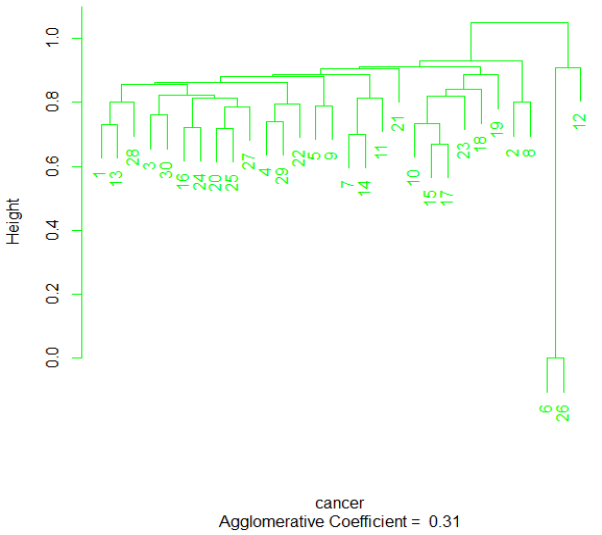

Figure 1. Dendrogram clustering plot of global pair-wise alignment between each pair sets of investigated proteins of the pancreatic cancer. The agglomerative coefficient (AC) is 0.31 for this analysis.

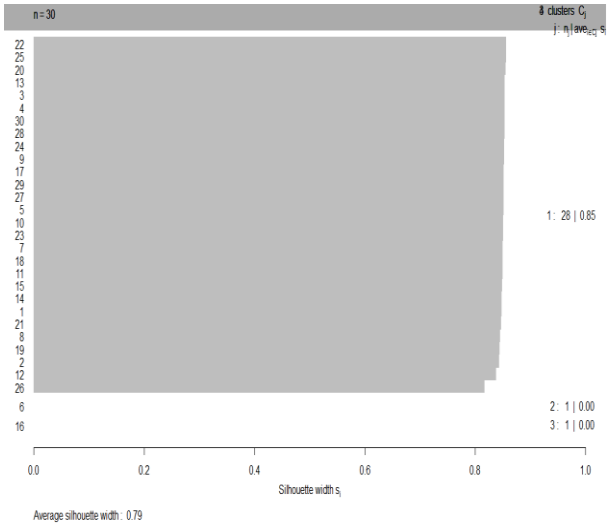

Figure 2. Silhouette plot presentation of global pair-wise alignment between each pair sets of investigated proteins of the pancreatic cancer, the agglomerative coefficient (AC) is 0.79 with k=3.

Pair-wise alignment among of pairs of these 30 proteins was performed and clustering method

Table 1. Proteins involved in pancreatic cancer achieving from Wiki pathway

row	UniProt Code	Gene Name	Protein Name	row	UniProt code	Gene Name	Protein Name
1	P62834	RAP1A	Ras-related protein Rap-1A	16	P19525	EIF2AK2	Interferon-induced, double-stranded RNA-activated protein kinase
2	P42345	MTOR	Serine/threonine-protein kinase mTOR	17	Q13625	TP53BP2	Apoptosis-stimulating of p53 protein 2
3	P25963	NFKBIA	NF-kappa-B inhibitor alpha	18	P10275	AR	Androgen receptor
4	O15111	CHUK	Inhibitor of nuclear factor kappa-B kinase subunit alpha	19	Q92793	CREBBP	CREB-binding protein
5	Q15078	CDK5R1	Cyclin-dependent kinase 5 activator 1	20	P06493	CDK1	Cyclin-dependent kinase 1
6	Q8N726	CDKN2A	Tumor suppressor ARF	21	P00533	EGFR	Epidermal growth factor receptor
7	P38936	CDKN1A	Cyclin-dependent kinase inhibitor 1	22	Q01094	E2F1	Transcription factor E2F1
8	P08183	ABCB1	Multidrug resistance protein 1	23	Q03468	ERCC6	DNA excision repair protein ERCC-6
9	P17936	IGFBP3	Insulin-like growth factor-binding protein 3	24	P30307	CDC25C	M-phase inducer phosphatase 3
10	P10636	MAPT	Microtubule-associated protein tau	25	O14757	CHEK1	Serine/threonine-protein kinase Chk1
11	P01034	CST3	Cystatin-C	26	P52657	GTF2A2	Transcription initiation factor IIA subunit 2
12	Q6LD43	GA	GA protein	27	P35354	PTGS2	Prostaglandin G/H synthase 2
13	P51948	MNAT1	CDK-activating kinase assembly factor MAT1	28	P24522	GADD45A	Growth arrest and DNA damage-inducible protein GADD45 alpha
14	P15531	NME1	Nucleoside diphosphate kinase A	29	P49959	MRE11A	Double-strand break repair protein MRE11A
15	Q8WUF5	PPP1R13L	RelA-associated inhibitor	30	O96017	CHEK2	Serine/threonine-protein kinase Chk2

was performed based on sequence comparison. Dendrogram clustering plot of protein sequences is presented as figure 1,2.

Agglomerative coefficient is 0.31. The similarity is the main concept for sequence evaluation. In figure 3, the interactions of 30 proteins with their neighbors are presented by the application of PSICQUIC source in Cytoscape software. To introduce the important key proteins in the network achieve by the use of Network Analyzer.

Protein-protein interactions are intrinsic to virtually every cellular process (38) and was applied for network relationship study and can make spatial proteins an attractive source of therapeutic targets (39). The integrated network

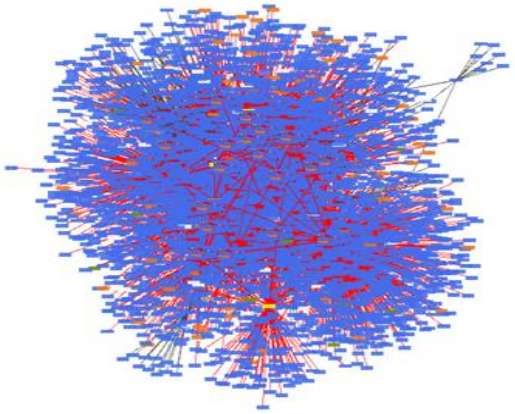

Figure 3. Protein-protein interaction network analysis of 30 proteins using Cytoscape v.3.2.1 software derived from Mentha, Reactome-Fls public databases.

was obtained from Reactome-Fls, MINT by the application of Proteomics Standard Initiative Common QUery InterfaCe (PSICQUIC) source. As it is shown in table 2, based on centrality parameters of network (Degree and Betweeness) Tumor suppressor ARF, Transcription factor E2F1, Inhibitor of nuclear factor kappa-B kinase subunit alpha, NF-kappa-B inhibitor alpha, Serine/threonine-protein kinase mTOR, Cyclin-dependent kinase 1, Cyclin-dependent kinase inhibitor 1, Androgen receptor, CREB-binding protein and Epidermal growth factor receptor are identified as hubs of network (table 2).

The extracted proteins are close distance with hubs. The biological process and pathway of closed proteins with hubs in the cluster was collected (table 3). Their biological process was brought from Quick GO and the origin pathway surveyed in Reactome database.

Table 2. Hub proteins with significant centrality values, based on two fundamental centrality properties Degree and Betweenness.

Protein name	degree	Betweenness centrally
Tumor suppressor ARF	422	0.05268923
Transcription factor E2F1	519	0.05897625
Inhibitor of nuclear factor kappa-B kinase subunit alpha	568	0.05389607
NF-kappa-B inhibitor alpha	612	0.05496298
Serine/threonine-protein kinase mTOR	664	0.07942783
Cyclin-dependent kinase 1	771	0.17460146
Cyclin-dependent kinase inhibitor 1	938	0.11365093
Androgen receptor	1015	0.13463114
CREB-binding protein	1244	0.17327638
Epidermal growth factor receptor	2291	0.44109984

Discussion

Pancreatic cancer is one of the aggressive malignancy with a very poor prognosis (40). Late diagnosis and lack of effective therapeutic for pancreatic cancer are one of the essential concerns in medicine. Therefore introducing biomarkers in early diagnosis or drug targets can be helpful in predicting progression and in time therapeutic proceeding.

Diagnostic biomarkers and therapeutic targets can be accelerated by targeting the specific hubs (proteins with the high degree) (41). Protein Networks analysis provide a model that elevate systems-level understanding of the mechanisms of diseases (42, 43) and discovering novel network-based biomarkers (44) to analysis therapeutic drugs and their targets (45-47). In fact, The importance of PPI analysis has been reported in cancer-related protein (41, 48).

Enrichment analysis methods have been developed for the investigation in the set of disease-related genes and find common relations and can be helpful in understanding of

Table 3. Introduction of closed protein hubs in sequence clustering and pathways that involved

Protein name	Number of Closed protein in cluster	Biological process of closed proteins in cluster	Pathway of closed proteins in cluster
Tumor suppressor ARF	12	Metabolic process; GO:0008152	Metabolism
regulation of transcription , DNA template GO:0006351	26	regulation of transcription , DNA template; GO:0006351	Gene expression
Transcription factor E2F1	29	DNA repair GO:0006281	DNA repair
Check point, G1/S transition of mitotic cell cycle GO:0000082	4	epithelial growth factor receptor signaling pathway GO:0007371	Signal transduction
Inhibitor of nuclear factor kappa-B kinase	29	DNA repair; GO:0006281	DNA repair
subunit alpha; GO:0007371	22	Check point, G1/S transition of mitotic cell cycle GO:0000082	Cell cycle
epithelial growth factor receptor signaling pathway			
NF-kappa-B inhibitor alpha	30	DNA damage check point; GO:000077	Cell cycle
Translocation GO:0000060			
Serine/threonine-protein kinase mTOR	8	G2/M transition of mitotic cell cycle; GO:000086	Cell cycle
Positive regulation of endothelial cell proliferation GO: 0001934			
Cyclin-dependent kinase 1	25	G2/M transition of mitotic cell cycle; GO:0000086	Cell cycle
G2/M transition of mitotic cell cycle GO:0000086			
Cyclin-dependent kinase inhibitor 1	14	negative regulation of cell proliferation; GO:0008285	Signal transduction
G1/S transition of mitotic cell cycle GO:0000082	11	positive regulation of cell proliferation; GO:0008284	Signal transduction
Androgen receptor	17	apoptotic process; GO: 0006915	Programmed cell death
regulation of transcription , DNA	15	apoptotic process; GO: 0006915	Programmed cell death
template	10	apoptotic process; GO: 0006915	Programmed cell death
GO:0006351	23	regulation of transcription , DNA template GO:0006351	Gene expression
CREB-binding protein	18	regulation of transcription , DNA template GO:0006351	Gene expression
regulation of transcription , DNA			
template	17	apoptotic process; GO: 0006915	Programmed cell death
	15	apoptotic process; GO: 0006915	Programmed cell death
	10	apoptotic process; GO: 0006915	Programmed cell death
GO:0006351	23	regulation of transcription , DNA template GO:0006351	Gene expression
Epidermal growth factor receptor	11	positive regulation of cell proliferation	Signal transduction
MAPK cascade	14	GO:0008284	Signal transduction
	7	negative regulation of cell proliferation	Cell cycle
GO:0000165	9	GO:0008285	Signal transduction
	5	G1/S transition of mitotic cell cycle	Signal transduction
	29	GO:0000082	DNA repair
	4	regulation of cell growth; GO:0001558	Signal transduction
	27	cell proliferation; GO:0006915	Signal transduction
	20,24,30,1,28,13	DNA repair; GO:0006281	Cell cycle
	16	epithelial growth factor receptor signaling	Signal transduction
	3	pathway; GO:0007371	------
	1	positive cell proliferation; GO: 0008284	Signal transduction
		G2/M transition of mitotic cell cycle GO:0000086	
		activation of MAPKK activity; GO:000628	
		Translocation; GO:0000060	
		activation of MAPK activity GO:000186	

the disease pathology mechanism. The use of annotation methods for example mapping genes /proteins by gene ontology [GO] can be helpful in understanding and gain a better view of biological features of the interest sets of protein (49). Cluster analysis can classify

proteins based on distinct similarities (28). Here related proteins are subdivided groups in via this method. In first, extracted proteins from Wiki Pathway in pancreatic cancer as shown in Table 1 were analyzed. Clustering analysis of the proteins according to sequence pair-wise alignment was done and PPI network was shown. According to proteins that involved in pancreatic cancer and reported in Wiki Pathway Androgen receptor, CREB-binding protein and epidermal growth factor receptor are most significant hubs but not in the high close relationship in sequence clustering. In this study, epidermal growth factor receptor has highest significant centrality values between hub proteins. Epidermal growth factor receptor (EGFR) is a member of the erbB/human epidermal growth factor receptor family of tyrosine kinases that transmit a growth-inducing signal to cells (50). Evidence suggests that over expression of EGFR is involved in the pathogenesis and progression of different carcinoma types and it able to induce cell transformation and metastasis, resistance to chemotherapy (51, 52). Overexpression of EGFR may be detected in up to 90% of pancreatic tumors (53). Another hub is Androgen Receptor that in previous studies have been reported the relationship of the androgen receptor (AR) with the pancreas carcinogenesis (54, 55). It has been suggested that The growth of pancreatic adenocarcinoma may be under the control of the sex steroid hormone testosterone there for this relationship may be acceptable (55, 56). The other hub is CREB protein that carries out its function by activating transcription via interaction with transcription factors. It plays critical roles in growth control, embryonic development and homeostasis by coupling chromatin remodeling (57). There have been relatively few studies addressing the pathobiology of pancreatic cancer and its association with CREB (58). In this study closed proteins in sequence for hubs was collected and their biological process information was extracted from Quick GO. The common pathways that pancreatic cancer hubs involved include of gene expression, cell cycle, signaling pathway (table 4). For example the most proteins that related with AR in sequence clustering, involved in signal transduction (table 4). Since, cell cycle and signal transduction are associated with control of cell growth and proliferation , they can play a significant role in tumorigenesis (59, 60).

The results show the closed proteins in sequence may participate in the same biological process. On the other hand, the most proteins with closed distance with hubs in clustering, regulate the events of cell cycle, apoptotic process and signal transduction. It also proven that regulation of cell cycle process (61, 62), signal transduction (63-65) and apoptotic process (66, 67) are related to pancreatic cancer. The knowledge about the complex pathways involved in cancer has strongly increased(68) and new therapeutic approaches based on this knowledge can develop(69). However, to unravel the possible role(s) of these proteins in pancreatic cancer, further investigations are needed.

Conclusion

The pathways that pancreatic cancer hubs involved in gene expression are included, cell cycle and signaling pathway. In this work, it has been visualized not only complex interactions

among the pancreatic cancer proteins involved but also comprehend their relative importance in the network based on sequence clustering. Cell cycle and signaling pathway have highlighted its importance in pancreatic cancer, as well as its potential as a therapeutic target in PC. The results show the closed proteins in sequence may participate in the same biological process. Such identification can promote a better understanding of the underlying disease process and also identify specific gene targets for therapy.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, et al. Cancer statistics, 2008. CA Cancer J Clin 2008;58:71-96.

2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.

3. Ghaneh P, Costello E, Neoptolemos JP. Biology and management of pancreatic cancer. Gut 2007;56:1134-52.

4. Chen R, Pan S, Brentnall TA, Aebersold R. Proteomic profiling of pancreatic cancer for biomarker discovery. Mol Cell Proteomics 2005;4:523-33.

5. Neoptolemos JP, Stocken DD, Dunn JA, Almond J, Beger HG, Pederzoli P, et al. Influence of resection margins on survival for patients with pancreatic cancer treated by adjuvant chemoradiation and/or chemotherapy in the ESPAC-1 randomized controlled trial. Ann Surg 2001;234:758-68.

6. Inoue S, Tezel E, Nakao A. Molecular diagnosis of pancreatic cancer. Hepato-gastroenterology. 2000;48:933-38.

7. Gutiérrez ML, Muñoz-Bellvis L, Abad Mdel M, Bengoechea O, González-González M, Orfao A, et al. Association between genetic subgroups of pancreatic ductal adenocarcinoma defined by high density 500 K SNP-arrays and tumor histopathology. PLoS One 2011;6:e22315.

8. Rizzato C, Campa D, Giese N, Werner J, Rachakonda PS, Kumar R, et al. Pancreatic cancer susceptibility loci and their role in survival. PLoS One 2011;6:e27921.

9. Oliveira-Cunha M, Newman WG, Siriwardena AK. Epidermal growth factor receptor in pancreatic cancer. Cancers 2011;3:1513-26.

10. Hruban RH, Goggins M, Parsons J, Kern SE. Progression model for pancreatic cancer. Clin Cancer Res 2000;6:2969-72.

11. Nowak NJ, Gaile D, Conroy JM, McQuaid D, Cowell J, Carter R, et al. Genome-wide aberrations in pancreatic adenocarcinoma. Cancer Genet Cytogenet 2005;161:36-50.

12. Amundadottir L, Kraft P, Stolzenberg-Solomon RZ, Fuchs CS, Petersen GM, Arslan AA, et al. Genome-wide association study identifies variants in the ABO locus associated with susceptibility to pancreatic cancer. Nature Gen 2009;41:986-90.

13. Jones S, Hruban RH, Kamiyama M, Borges M, Zhang X, Parsons DW, et al. Exomic sequencing identifies PALB2 as a pancreatic cancer susceptibility gene. Science 2009;324:217.

14. Murphy KM, Brune KA, Griffin C, Sollenberger JE, Petersen GM, Bansal R, et al. Evaluation of Candidate Genes MAP2K4, MADH4, ACVR1B, and BRCA2 in Familial Pancreatic Cancer Deleterious BRCA2 Mutations in 17%. Can Res 2002;62:3789-93.

15. Su GH, Hruban RH, Bansal RK, Bova GS, Tang DJ, Shekher MC, et al. Germline and somatic mutations of the STK11/LKB1 Peutz-Jeghers gene in pancreatic and biliary cancers. Am J Pathol 1999;154:1835-40.

16. Lynch HT, Brand RE, Hogg D, Deters CA, Fusaro RM, Lynch JF, et al. Phenotypic variation in eight extended CDKN2A germline mutation familial atypical multiple mole melanoma–pancreatic carcinoma–prone families. Cancer 2002;94:84-96.

17. Chen J-H, Ni R-Z, Xiao M-B, Guo J-G, Zhou J-W. Comparative proteomic analysis of differentially expressed proteins in human pancreatic cancer tissue. Hepatobiliary Pancreat Dis Int 2009; 8:193-200.

18. Xue A, Scarlett C, Chung L, Butturini G, Scarpa A, Gandy R, et al. Discovery of serum biomarkers for pancreatic adenocarcinoma using proteomic analysis. Br J Cancer 2010;103:391-400.

19. Takano S, Sogawa K, Yoshitomi H, Shida T, Mogushi K, Kimura F, et al. Increased circulating cell signalling phosphoproteins in sera are useful for the detection of pancreatic cancer. Br J Cancer 2010;103:223-31.

20. Weeks ME, Hariharan D, Petronijevic L, Radon TP, Whiteman HJ, Kocher HM, et al. Analysis of the urine

proteome in patients with pancreatic ductal adenocarcinoma. Proteomics Clin Appl 2008;2: 1047-57.

21. Ideker T, Sharan R. Protein networks in disease. Genome Res2008;18:644-52.

22. King AD, Pržulj N, Jurisica I. Protein complex prediction via cost-based clustering. Bioinformatics 2004;20:3013-20.

23. Sigkdd A. Data mining curriculum. 2012.

24. Consortium GO. The Gene Ontology (GO) database and informatics resource. Nucleic Acids Res 2004;32D258-D61.

25. Consortium GO. Creating the gene ontology resource: design and implementation. Genome Res 2001;11:1425-33.

26. Jiang P, Singh M. SPICi: a fast clustering algorithm for large biological networks. Bioinformatics 2010;26:1105-11.

27. Rezaei-Tavirani M, Zamanian-Azodi M, Rajabi S, Masoudi-Nejad A, Rostami-Nejad M, Rahmatirad S. Protein Clustering and Interactome Analysis in Parkinson and Alzheimer's Diseases. Arch Iran Med 2016;19:101-109.

28. Daraselia N, Yuryev A, Egorov S, Mazo I, Ispolatov I. Automatic extraction of gene ontology annotation and its correlation with clusters in protein networks. BMC Bioinformatics 2007;8:243.

29. Nia YZ, Majd HA, Azodi M, Khayyer N. Using partitioning and non-partitioning clustering algorithms for included proteins sequences in esophagus, stomach and colon cancer. Journal of Paramedical Sciences. 2011;2: 22-29.

30. Loewenstein Y, Portugaly E, Fromer M, Linial M. Efficient algorithms for accurate hierarchical clustering of huge datasets: tackling the entire protein space. Bioinformatics 2008;24:i41-i9.

31. Binns D, Dimmer E, Huntley R, Barrell D, O'Donovan C, Apweiler R. QuickGO: a web-based tool for Gene Ontology searching. Bioinformatics 2009;25:3045-46.

32. Huang DW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 2009;37:1-13.

33. Liu J, Rost B. Domains, motifs and clusters in the protein universe. Curr Opin Chem Biol 2003;7:5-11.

34. D'Haeseleer P. How does gene expression clustering work? Nat Biotechnol 2005;23:1499-501.

35. SERRES MH, RILEY M. MultiFun, a multifunctional classification scheme for Escherichia coli K-12 gene products. Microb Comp Genomics 2000;5:205-22.

36. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene Ontology: tool for the unification of biology. Nature Gen 2000;25:25-29.

37. Consortium GO. The Gene Ontology in 2010: extensions and refinements. Nucleic Acids Res 2010;38D331-D5.

38. Groll N, Emele F, Poetz O, Rothbauer U. Towards multiplexed protein–protein interaction analysis using protein tag-specific nanobodies. J Proteomics 2015;127:289-99.

39. Bultinck J, Lievens S, Tavernier J. Protein-protein interactions: network analysis and applications in drug discovery. Curr Pharm Des 2012;18:4619-29.

40. Parker SL, Tong T, Bolden S, Wingo PA. Cancer statistics, 1997. CA Cancer J Clin 1997;47:5-27.

41. Zamanian-Azodi M, Rezaei-Tavirani M, Rahmati-Rad S, Hasanzadeh H, Tavirani MR, Seyyedi SS. Protein-Protein Interaction Network could reveal the relationship between the breast and colon cancer. Gastroenterol Hepatol bed bench 2015;8:215-24.

42. Goh K-I, Cusick ME, Valle D, Childs B, Vidal M, Barabasi A-L. The human disease network. Proc Natl Acad Sci U S A 2007;104:8685-90.

43. Yang X, Deignan JL, Qi H, Zhu J, Qian S, Zhong J, et al. Validation of candidate causal genes for obesity that affect shared metabolic pathways and networks. Nature Gen 2009;41:415-23.

44. Re M, Valentini G. Network-based drug ranking and repositioning with respect to DrugBank therapeutic categories. IEEE/ACM Trans Comput Biol Bioinform 2013;10:1359-71.

45. Yıldırım MA, Goh K-I, Cusick ME, Barabási A-L, Vidal M. Drug—target network. Nature Biotechnol 2007;25:1119-26.

46. Winter C, Kristiansen G, Kersting S, Roy J, Aust D, Knösel T, et al. Google goes cancer: improving outcome prediction for cancer patients by network-based ranking of marker genes. PLoS Comput Biol. 2012;8:e1002511.

47. Gottlieb A, Stein GY, Ruppin E, Sharan R. PREDICT: a method for inferring novel drug indications with

application to personalized medicine. Mol Sys Biol 2011;7:496.

48. Zali H, Rezaei Tavirani M. Meningioma protein-protein interaction network. Arch Iran Med 2014;17:262-72.

49. Tipney H, Hunter L. An introduction to effective use of enrichment analysis software. Hum Genomics 2010;4:202-206.

50. Carpenter G, Cohen S. Epidermal growth factor. J Biol Chem 1990;265:7709-12.

51. Normanno N, De Luca A, Bianco C, Strizzi L, Mancino M, Maiello MR, et al. Epidermal growth factor receptor (EGFR) signaling in cancer. Gene 2006;366:2-16.

52. De Jong KP, Stellema R, Karrenbeld A, Koudstaal J, Gouw AS, Sluiter WJ, et al. Clinical relevance of transforming growth factor α, epidermal growth factor receptor, p53, and Ki67 in colorectal liver metastases and corresponding primary tumors. Hepatology 1998;28:971-79.

53. Troiani T, Martinelli E, Capasso A, Morgillo F, Orditura M, De Vita F, et al. Targeting EGFR in pancreatic cancer treatment. Curr Drug targets. 2012;13:802-10.

54. Okitsu K, Kanda T, Imazeki F, Yonemitsu Y, Ray RB, Chang C, et al. Involvement of interleukin-6 and androgen receptor signaling in pancreatic cancer. Genes Cancer 2010;1:859-67.

55. Greenway BA. Androgen Receptor-Blocking Agents. Drugs Aging 2000;17:161-63.

56. Andrén-Sandberg Å, Hoem D, Bäckman PL. Other risk factors for pancreatic cancer: hormonal aspects. Ann Oncol 1999;10:S131-S5.

57. Ogryzko VV, Schiltz RL, Russanova V, Howard BH, Nakatani Y. The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell 1996;87:953-59.

58. Arensman MD, Telesca D, Lay AR, Kershaw KM, Wu N, Donahue TR, et al. The CREB-binding protein inhibitor ICG-001 suppresses pancreatic cancer growth. Mol Cancer ther 2014;13:2303-14.

59. Li M, Becnel LS, Li W, Fisher WE, Chen C, Yao Q. Signal transduction in human pancreatic cancer: roles of transforming growth factor beta, somatostatin receptors, and other signal intermediates. Arch Immunol Ther Exp 2005;53:381-87.

60. Abramson MA, Jazag A, van der Zee JA, Whang EE. The molecular biology of pancreatic cancer. Gastrointest Cancer Res2007;1:S7-S12.

61. Kokkinakis DM, Liu X, Neuner RD. Modulation of cell cycle and gene expression in pancreatic tumor cell lines by methionine deprivation (methionine stress): implications to the therapy of pancreatic adenocarcinoma. Mol Cancer ther 2005;41338-48.

62. Cattaneo M, Fontanella E, Canton C, Delia D, Biunno I. SEL1L affects human pancreatic cancer cell cycle and invasiveness through modulation of PTEN and genes related to cell-matrix interactions. Neoplasia 2005;7:1030-38.

63. Sun X, Li Z, Li M. Signal Transduction Therapy in Pancreatic Cancer. Current Signal Transduction Therapy 2014;9:78-82.

64. Li M, Becnel LS, Li W, Fisher WE, Chen C, Yao Q. Signal transduction in human pancreatic cancer: roles of transforming growth factor beta, somatostatin receptors, and other signal intermediates. Arch Immunol Ther Exp (Warsz). 2005;53:381-87.

65. Egami H, Tan X, Kurizaki T, Ishikawa S, Hayashi N, Hirota M, et al., editors. Analysis of the signal transduction pathway in relation to invasion and metastasis in pancreatic cancer. International Congress Series; 2003: Elsevier.

66. Westphal S, Kalthoff H. Apoptosis: targets in pancreatic cancer. Mol Cancer2003;2:6.

67. Hamacher R, Schmid RM, Saur D, Schneider G. Apoptotic pathways in pancreatic ductal adenocarcinoma. Mol Cancer 2008;7:64.

68. Grant S. Cotargeting survival signaling pathways in cancer. J Clin Invest 2008;118:3003-306.

69. Garattini S. New approaches to cancer therapy. Ann Oncol 2003;14:813-16.