Pathol. Oncol. Res. (2014) 20:853–858
DOI 10.1007/s12253-014-9764-z

RESEARCH

Screening of Critical Genes in Lung Adenocarcinoma via Network Analysis of Gene Expression Profile

Ping Huang · Kejian Cao · Heng Zhao

Received: 18 January 2014 / Accepted: 14 March 2014 / Published online: 26 May 2014

Abstract Biomarker discovery is of great importance in diagnosis and treatment of diseases. In present study, a number of differentially expressed genes (DEGs) were identified for lung adenocarcinoma via comparative analysis of gene expression data. A gene expression core signature was generated for four types of lung adenocarcinoma (EGFR-mutated, KRAS-mutated, ALK-mutated and triple-negative adenocarcinoma). Functional enrichment analysis with DAVID tools revealed that up-regulated genes were mainly associated with cell cycle while down-regulated genes were mainly involved in vasculature development and cell adhesion. Then it was used to retrieve relevant small molecule drugs with Connectivity map and trichostatin A was predicted to be the top candidate drug for treatment of lung cancer. Network clustering was performed with MCL in cytoscape to identify sub-networks and several hub genes were obtained: CDC25C, ICT1, TK1 and EZH2. These genes play important roles in the progression of lung cancer and some have been suggested as potential biomarkers. Therefore, our findings are beneficial in deepening the understandings about the pathogenesis and providing directions for future researches.

Keywords Lung adenocarcinoma · Gene expression profile · Differentially expressed gene · Gene expression core signature · Functional enrichment analysis · Network clustering

P. Huang · K. Cao · H. Zhao (✉)
Department of Thoracic Surgery, Shanghai Chest Hospital, Shanghai Jiao Tong University, NO.241 Huaihaixi Road, Shanghai 200030, People's Republic of China
e-mail: H_zhang28@163.com

Introduction

Lung cancer is the leading cause of cancer-related death in the world. Adenocarcinoma, which accounts for more than 50 % of non-small-cell lung cancers (NSCLC), is the most frequent type and thus was investigated in present study. Previous studies have discovered at least 3 majorpathways participating in the development of lung adenocarcinoma [1–5]. A considerable percentage (30–60 %) of lung adenocarcinoma develops through acquisition of mutations either in the *EGFR, KRAS*, or *ALK* genes in a mutually exclusive manner, and the remaining lung adenocarcinoma, that is, those without *EGFR, KRAS*, and *ALK* mutations (herein designated "triple-negative adenocarcinoma"), develops with mutations of several other genes. *HER2*, *BRAF*, etc. are also known to be mutated mutually exclusively with the *EGFR, KRAS*, and *ALK* genes.

In present study, we compared gene expression profile of lung adenocarcinoma (EGFR-mutated, KRAS-mutated, ALK-mutated and triple-negative adenocarcinoma separately) with normal lung tissue and identified a gene expression core signature. Based on this coresignature, we predicted potential drugs that might have antitumor effects forlung cancer. Besides, we integrated protein-protein interaction and gene-gene co-expression to construct protein-interaction networks for each type of lung adenocarcinoma.

Materials and Methods

Microarray Data

Microarray data set GSE31210 was downloaded from GEO, including 20 normal lung tissue samples and 226 lung

Table 1 Functional enrichment analysis results for the core signature

	Category	Term	Count	*P*-value	FDR
Upregulated	GOTERM_BP_FAT	GO:0000280 ~ nuclear division	20	5.53E-14	5.01E-11
	GOTERM_BP_FAT	GO:0007067 ~ mitosis	20	5.53E-14	5.01E-11
	GOTERM_BP_FAT	GO:0000087 ~ M phase of mitotic cell cycle	20	7.67E-14	3.48E-11
	GOTERM_BP_FAT	GO:0048285 ~ organelle fission	20	1.15E-13	3.47E-11
	GOTERM_BP_FAT	GO:0000279 ~ M phase	21	8.16E-12	1.85E-09
	GOTERM_BP_FAT	GO:0000278 ~ mitotic cell cycle	22	8.55E-12	1.55E-09
	GOTERM_BP_FAT	GO:0022403 ~ cell cycle phase	22	7.05E-11	1.07E-08
	GOTERM_BP_FAT	GO:0051301 ~ cell division	17	6.22E-09	8.06E-07
	GOTERM_BP_FAT	GO:0022402 ~ cell cycle process	22	1.89E-08	2.15E-06
	GOTERM_BP_FAT	GO:0007059 ~ chromosome segregation	9	5.33E-07	5.37E-05
	GOTERM_BP_FAT	GO:0007049 ~ cell cycle	23	9.41E-07	8.53E-05
Down-regulated	GOTERM_BP_FAT	GO:0001944 ~ vasculature development	29	2.87E-12	5.64E-09
	GOTERM_BP_FAT	GO:0001568 ~ blood vessel development	28	9.54E-12	9.39E-09
	GOTERM_BP_FAT	GO:0007155 ~ cell adhesion	43	6.52E-09	4.28E-06
	GOTERM_BP_FAT	GO:0022610 ~ biological adhesion	43	6.68E-09	3.29E-06
	GOTERM_BP_FAT	GO:0048514 ~ blood vessel morphogenesis	21	6.66E-08	2.62E-05
	GOTERM_BP_FAT	GO:0001525 ~ angiogenesis	17	2.35E-07	7.72E-05
	GOTERM_BP_FAT	GO:0042127 ~ regulation of cell proliferation	41	1.25E-06	3.51E-04

Only biological processes with a false discovery rate (FDR) less than 0.001 were shown in the list. Count is the number of genes annotated by the corresponding term. The *p*-values associated with each terms inside the clusters is *p*-values by the Fisher Exact Test which represent the "degree of enrichment" of the annotation term with the input gene list. Benjamini FDR *q*-value is the correction for multiple comparison

adenocarcinoma samples. The status of EGFR, KRAS and ALK mutations have been examined for all tumors and provided by original authors. Gene expression profiling was performed by Affymetrix Human Genome U133 Plus 2.0 Array. Gene expression intensities were calculated using custom chip description file [6] by RMA [7].

Identification of Differentially Expressed Genes

Four subtypes of lung cancer were included in this study: EGFR-mutated, KRAS-mutated, ALK-fusion and triple-negative (TN). Normal lung tissue was used as the control and Student's *t* test was applied to examine the significance of alteration in gene expression. Genes with *p*-value less than 0.001 wereconsidered as significantand added intoprotein-protein interaction networks. In addition, significantly alteredgeneswith a fold-change of at least 2 in all four subtypes were regarded as components of the gene expression core signature of lung cancer.

Functional enrichment analysis was performed for the core lung cancer gene expression signature with DAVID [8], which can provide significantly over-represented Gene Ontology biological processes in the query gene list.

Drug Prediction Using Connectivity Map

Potential drugs were retrieved in Connectivity map (CMap) [9] with the core signature. CMap is an in-silico method to predict potential drugs that could possibly reverse, or induce, the biological state encoded in particular gene expression signatures. It provides a collection of more than 7,000 genome-wide transcriptional expression data from cultured human cells treated with 1,309 bioactive small molecules. Gene expression profiles were organized into instances which represent a treatment and control pair and the list of genes ordered by their extent of differential expression between this treatment and control pair. The query gene signature is then compared to each rank-ordered list to determine whether up-regulated query genes tend to appear near the top of the list and down-regulated query genes near the bottom ("positive connectivity") or vice versa ("negative connectivity"), yielding a "connectivity score" ranging from −1 to 1. A high positive connectivity score indicates that the corresponding perturbagen induced the expression of the query signature whilea high negative connectivity score indicates that the corresponding perturbagen reversed the expression of the query signature. All instances in the database are then ranked according to their connectivity scores; those at the top are most strongly correlated to the query signature, and those at

Table 2 Top 20 chemical compounds identified by CMap

Rank	CMap name	Mean	*N*	Enrichment	*P*
1	Trichostatin A	−0.443	182	−0.346	0
2	Vorinostat	−0.56	12	−0.59	0.0002
3	8-azaguanine	−0.872	4	−0.895	0.00022
4	Apigenin	−0.765	4	−0.886	0.00038
5	Resveratrol	−0.696	9	−0.641	0.00044
6	Chenodeoxycholic acid	0.615	4	0.864	0.00046
7	Podophyllotoxin	0.692	4	0.86	0.0005
8	3-acetamidocoumarin	0.679	4	0.858	0.00054
9	Atractyloside	0.627	5	0.806	0.00062
10	Prestwick-1084	−0.709	4	−0.841	0.00117
11	Phenoxybenzamine	−0.735	4	−0.839	0.00119
12	Genistein	0.285	17	0.44	0.00188
13	Thiostrepton	−0.722	4	−0.823	0.00189
14	Thioguanosine	−0.742	4	−0.821	0.00197
15	Diethylstilbestrol	0.53	6	0.698	0.00205
16	Gentamicin	0.604	4	0.81	0.00243
17	Terazosin	0.614	4	0.798	0.00318
18	Methazolamide	−0.66	4	−0.798	0.00332
19	Quinpirole	0.676	4	0.791	0.00368
20	GW-8510	−0.648	4	−0.791	0.00384

Mean: the arithmetic mean of the connectivity scores for corresponding instances. Instance represents treatment and control pair and the list of probe sets ordered by their extent of differential expression between this treatment and control pair. A high positive mean indicates that the corresponding perturbagen induced the expression of the query signature. A high negative mean indicates that the corresponding perturbagen reversed the expression of the query signature. *N*: the number of instances. *Enrichment*: A measure of the enrichment of those instances in the order list of all instances. *P*: An estimate of the likelihood that the enrichment of a set of instances in the list of all instances in a given result would be observed by chance

the bottom are most strongly anticorrelated. Gene symbols for the coresignature were converted into Affymetrix probeset IDs as cMap requires.

Integration of Protein-Protein Interaction and Gene-Gene Co-expression Network

Human protein-protein interaction (PPI) information was collected from three public databases: MINT [10], BioGrid [11] and HPRD [12]. Only the interactionscollected by at least two databases were used in our analysis. For each two genes that formed an interaction, the correlation of their expression profile was calculated in each subtype of lung cancer and normal lung tissues separately (Pearson's correlation coefficient). For each subtype, interactions with positive correlation (Pearson's $r>0.3$) and p-value less than 0.01 in that subtype but larger than 0.05 in normal tissues were included in the subtype specific PPI network. Finally, only significantly altered genes were retained in each PPI network. Network clustering was performed using MCL [13] in cytoscape [14] to identify sub-networks.

Results

Gene Expression Core Signature of Lung Cancer

A total of 153 up-regulated genes and 435 down-regulated genes were included in the gene expression core signature. DAVID revealed that up-regulated genes were mainly associated with cell cycle whiledown-regulated genes were mainly involved invasculature developmentand cell adhesion (Table 1).

Potential Drugs Predicted by CMap

TrichostatinA(TSA),vorinostat,8-azaguanine,apigenin and resveratrol were predicted by cmap as the top five chemical compounds that might be used to treat lung cancer (Table 2). TSA was a histone deacetylase inhibitor and it was reported that co-treatment of lung cancer A549 cells with docetaxel or erlotinib synergistically inhibited cell proliferation, induced apoptosis, and caused cell cycle delay at the G2/M transition [15].

Lung Cancer Subtype-Specific PPI Network

PPI network for the ALK-fusion lung cancer was relatively simple. MCL-based network clustering revealed a CDC25C-centered PPI sub-network (Fig. 1). For EGFR-mutated, KRAS-mutated and TN lung cancers, ICT1 was found as a major hub gene but the ICT1-centered network showed rewiring in different subtypes (Fig. 2). Similarly, TK1-centered sub-network and EZH2-centered sub-network also showed rewiring in different subtypes (Figs. 3 and 4). Top ten sub-networks for EGFR-mutated, KRAS-mutated and TN lung cancers were provided in supplementary figures.

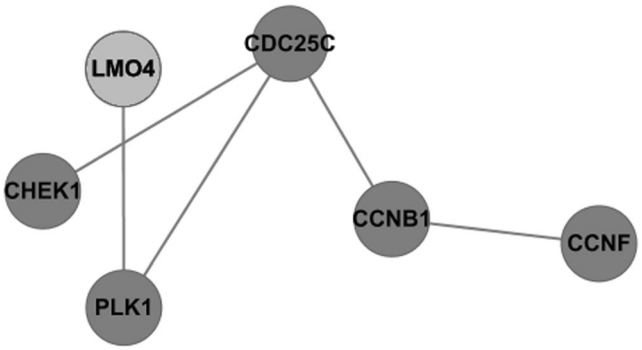

Fig. 1 A CDC25C-centered sub-network found in ALK-fusion lung adenocarcinomas. *Dark nodes* represent genes upregulated in lung cancers while *gray nodes* represent genes down-regulated in lung cancers

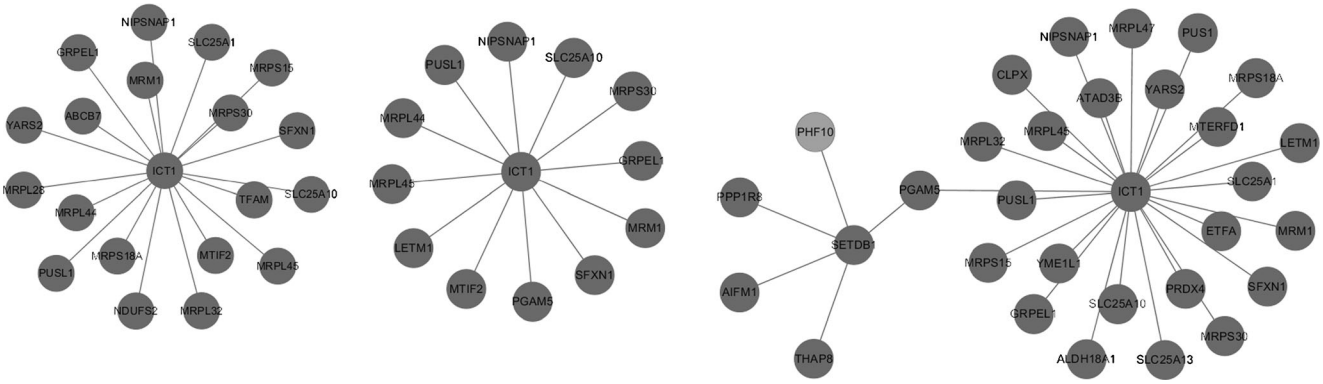

Fig. 2 ICT1-centered sub-networks found in EGFR-mutated (*left*), KRAS-mutated (*middle*) and triple-negative lung cancers (*right*). *Dark nodes* represent genes upregulated in lung cancers while *gray nodes* represent genes down-regulated in lung cancers

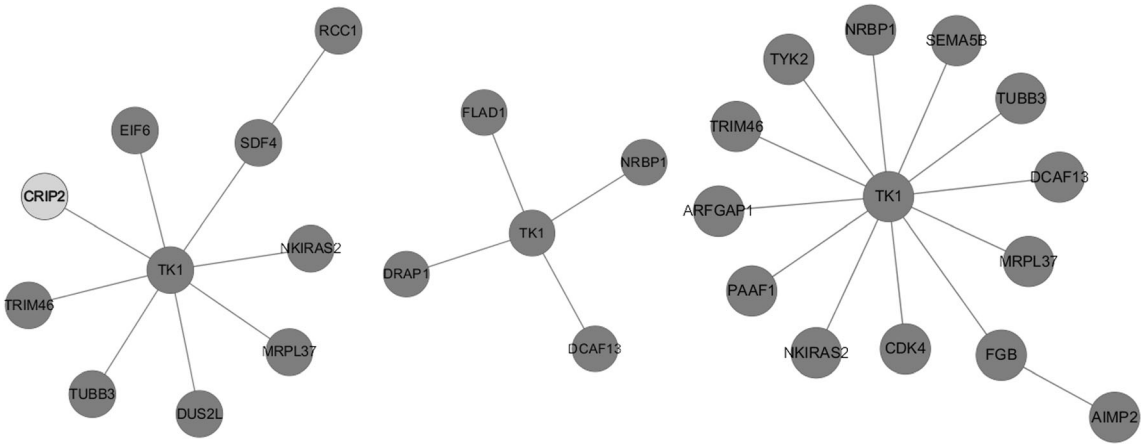

Fig. 3 TK1-centered sub-networks found in EGFR-mutated (*left*), KRAS-mutated (*middle*) and triple-negative lung cancers (*right*). *Dark nodes* represent genes upregulated in lung cancers while *gray nodes* represent genes down-regulated in lung cancers

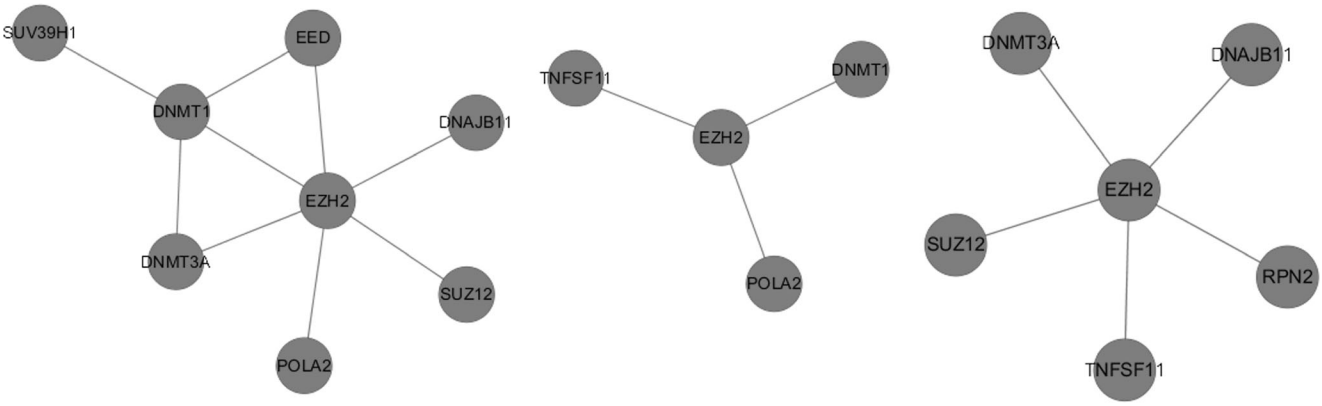

Fig. 4 EZH2-centered sub-networks found in EGFR-mutated (*left*), KRAS-mutated (*middle*) and triple-negative lung cancers (*right*). *Dark nodes* represent genes upregulated in lung cancers while *blue nodes* represent genes down-regulated in lung cancers

Discussion

In present study, a range of DEGs were revealed for lung cancer through comparative analysis of gene expression data. In order to discover key genes, network analysis was carried out for the DEGs and several hub genes were identified: cell division cycle 25C (CDC25C), immature colon carcinoma transcript 1 (ICT1), enhancer of zeste homolog 2 (EZH2) and thymidine kinase 1 (TK1).

TK1 has been suggested as a biomarker in many solid cancers [16, 17]. Korkmaz et al. determine serum TK1 activity by ELISA method and find that the serum TK1 level in patients with metastatic NSCLC is an independent prognostic predictor of overall survival [18]. Similarly, Xu et al. find that high thymidine kinase 1 (TK1) expression is a predictor of poor survival in patients with lung adenocarcinoma [19]. It proved the reliability of our methods in identifying key genes in the pathogenesis of lung cancer. Besides, its interactors were worthy of further study to fully disclose the underlying mechanisms and develop potential treatments.

CDC25C plays a key role in the regulation of cell division. It can direct dephosphorylation of cyclin B-bound CDC2 and trigger entry into mitosis [20]. It can also be down-regulated by tumor suppressor protein p53 [21]. Carmazzi et al. report that nadroparin inhibits proliferation of A549 cells by inducing G(2)/M phase cell-cycle arrest that is dependent on the Cdc25C pathway [22]. The study by Liet al suggest that the β-elemene-enhanced inhibitory effect of cisplatin on lung carcinoma cell proliferation is regulated by a CHK2-mediated CDC25C/CDC2/cyclin B1 signaling pathway and leads to the blockade of cell cycle progression at G(2)/M [23]. Therefore, it might be a good drug target to develop lung cancer therapy.

The ICT1 is originally discovered by comparison of gene expressionsbetween undifferentiated and differentiated HT29-D4 human colon carcinoma cells [24, 25]. Its mRNA is strongly downregulated during in vitro differentiation of HT29-D4 cells. Handa et al. indicate that knockdown of ICT1 results in apoptotic cell death with a decrease in mitochondrial membrane potential and mass. In addition, cytochrome c oxidase activity in ICT1 knockdown cells is decreased by 35 % compared to that in control cells. These results indicate that ICT1 function is essential for cell vitality and mitochondrial function [26]. Richter et al. also report that ICT1 is an essential mitochondrial protein and an integral component of the human mitoribosome. They speculate that ICT1 may be essential for hydrolysis of prematurely terminated peptidyl-tRNA moieties in stalled mitoribosomes [27]. Our analysis showed that ICT1 was a hub gene for the three different types of lung cancer. Therefore, we considered that it might worth further investigations to fully characterize its role.

EZH2 presents histone methyltransferase (HMT) activity, and it's found to be overexpressed in malignant tumors [28–30]. Cao et al. confirm the upregulation of EZH2 in NSCLC cells compared with normal human bronchial epithelial cells by western blot assay [31]. Upon EZH2 knockdown using small interfering RNA (siRNA), they observe that the proliferation, anchorage-independent growth and invasion of NSCLC cells are remarkably suppressed with profound induction of G1 arrest. In colorectal cancer, Linet al.find that knockdown of EZH2 significantly reduces cell invasion and secretion of matrix metalloproteinases 2/9 (MMP2/9) in in-vitro studies [32]. They further identifies VDR as a target gene of EZH2 and suggests that EZH2 expression may be directly regulated by STAT3 [32]. MicroRNA-101 exerts tumor-suppressive functions in NSCLC through directly targeting enhancer of EZH2 [33]. These findings suggest modulation of its expression may be a way to treat lung cancer.

Overall, DEGs identified in our study, especially the four hub genes were beneficial in strengthening the knowledge about lung cancer. The small molecule drugs predicted by cMap also could be a good guidance for future researches.

Conflict of Interest The authors report no conflicts of interest.

References

1. Bronte G, Rizzo S, La Paglia L, Adamo V, Siragusa S, Ficorella C, Santini D, Bazan V, Colucci G, Gebbia N, Russo A (2010) Driver mutations and differential sensitivity to targeted therapies: a new approach to the treatment of lung adenocarcinoma. Cancer Treat Rev 36(Suppl 3):S21–29. doi:10.1016/S0305-7372(10)70016-5
2. Gerber DE, Minna JD (2010) ALK inhibition for non-small cell lung cancer: from discovery to therapy in record time. Cancer Cell 18(6): 548–551
3. Herbst RS, Heymach JV, Lippman SM (2008) Lung cancer. N Engl J Med 359(13):1367–1380. doi:10.1056/NEJMra0802714
4. Janku F, Stewart DJ, Kurzrock R (2010) Targeted therapy in non-small-cell lung cancer–is it becoming a reality? Nat Rev Clin Oncol 7(7):401–414. doi:10.1038/nrclinonc.2010.64
5. Pao W, Girard N (2011) New driver mutations in non-small-cell lung cancer. Lancet Oncol 12(2):175–180. doi:10.1016/S1470-2045(10)70087-5
6. Dai M, Wang P, Boyd AD, Kostov G, Athey B, Jones EG, Bunney WE, Myers RM, Speed TP, Akil H, Watson SJ, Meng F (2005) Evolving gene/transcript definitions significantly alter the interpretation of GeneChip data. Nucleic Acids Res 33(20):e175
7. Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP (2003) Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 4(2):249–264. doi:10.1093/biostatistics/4.2.249
8. da Huang W, Sherman BT, Lempicki RA (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4(1):44–57
9. Lamb J, Crawford ED, Peck D, Modell JW, Blat IC, Wrobel MJ, Lerner J, Brunet JP, Subramanian A, Ross KN, Reich M, Hieronymus H, Wei G, Armstrong SA, Haggarty SJ, Clemons PA, Wei R, Carr SA, Lander ES, Golub TR (2006) The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. Science 313(5795):1929–1935

10. Licata L, Briganti L, Peluso D, Perfetto L, Iannuccelli M, Galeota E, Sacco F, Palma A, Nardozza AP, Santonico E, Castagnoli L, Cesareni G (2012) MINT, the molecular interaction database: 2012 update. Nucleic Acids Res 40(Database issue):D857–861. doi:10.1093/nar/gkr930
11. Chatr-Aryamontri A, Breitkreutz BJ, Heinicke S, Boucher L, Winter A, Stark C, Nixon J, Ramage L, Kolas N, O'Donnell L, Reguly T, Breitkreutz A, Sellam A, Chen D, Chang C, Rust J, Livstone M, Oughtred R, Dolinski K, Tyers M (2013) The BioGRID interaction database: 2013 update. Nucleic Acids Res 41(D1):D816–823. doi:10.1093/nar/gks1158
12. Keshava Prasad TS, Goel R, Kandasamy K, Keerthikumar S, Kumar S, Mathivanan S, Telikicherla D, Raju R, Shafreen B, Venugopal A, Balakrishnan L, Marimuthu A, Banerjee S, Somanathan DS, Sebastian A, Rani S, Ray S, Harrys Kishore CJ, Kanth S, Ahmed M, Kashyap MK, Mohmood R, Ramachandra YL, Krishna V, Rahiman BA, Mohan S, Ranganathan P, Ramabadran S, Chaerkady R, Pandey A (2009) Human Protein Reference Database–2009 update. Nucleic Acids Res 37(Database issue):D767–772. doi:10.1093/nar/gkn892
13. Enright AJ, Van Dongen S, Ouzounis CA (2002) An efficient algorithm for large-scale detection of protein families. Nucleic Acids Res 30(7):1575–1584
14. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13(11):2498–2504
15. Zhang QC, Jiang SJ, Zhang S, Ma XB (2012) Histone deacetylase inhibitor trichostatin A enhances anti-tumor effects of docetaxel or erlotinib in A549 cell line. Asian Pac J Cancer Prev 13(7):3471–3476
16. Henry CJ (2010) Biomarkers in veterinary cancer screening: applications, limitations and expectations. Vet J 185(1):10–14
17. Chen ZH, Huang SQ, Wang Y, Yang AZ, Wen J, Xu XH, Chen Y, Chen QB, Wang YH, He E (2011) Serological thymidine kinase 1 is a biomarker for early detection of tumours—a health screening study on 35,365 people, using a sensitive chemiluminescent dot blot assay. Sensors 11(12):11064–11080
18. Korkmaz T, Seber S, Okutur K, Basaran G, Yumuk F, Dane F, Ones T, Polat O, Madenci OC, Demir G, Turhal NS (2013) Serum thymidine kinase 1 levels correlates with FDG uptake and prognosis in patients with non small cell lung cancer. Biomarkers 18(1):88–94. doi:10.3109/1354750X.2012.738250
19. Xu Y, Shi QL, Ma H, Zhou H, Lu Z, Yu B, Zhou X, Eriksson S, He E, Skog S (2012) High thymidine kinase 1 (TK1) expression is a predictor of poor survival in patients with pT1 of lung adenocarcinoma. Tumour Biol 33(2):475–483. doi:10.1007/s13277-011-0276-0
20. Peng C-Y, Graves PR, Thoma RS, Wu Z, Shaw AS, Piwnica-Worms H (1997) Mitotic and G2 checkpoint control: regulation of 14-3-3 protein binding by phosphorylation of Cdc25C on serine-216. Science 277(5331):1501–1505
21. Krause K, Haugwitz U, Wasner M, Wiedmann M, Mössner J, Engeland K (2001) Expression of the cell cycle phosphatase cdc25C is down-regulated by the tumor suppressor protein p53 but not by p73. Biochem Biophys Res Commun 284(3):743–750
22. Carmazzi Y, Iorio M, Armani C, Cianchetti S, Raggi F, Neri T, Cordazzo C, Petrini S, Vanacore R, Bogazzi F, Paggiaro P, Celi A (2012) The mechanisms of nadroparin-mediated inhibition of proliferation of two human lung cancer cell lines. Cell Prolif 45(6):545–556. doi:10.1111/j.1365-2184.2012.00847.x
23. Li QQ, Wang G, Huang F, Li JM, Cuff CF, Reed E (2013) Sensitization of lung cancer cells to cisplatin by beta-elemene is mediated through blockade of cell cycle progression: antitumor efficacies of beta-elemene and its synthetic analogs. Med Oncol 30(1):488. doi:10.1007/s12032-013-0488-9
24. van Belzen N, Diesveld MP, van der Made AC, Nozawa Y, Dinjens WN, Vlietstra R, Trapman J, Bosman FT (1995) Identification of mRNAs that show modulated expression during colon carcinoma cell differentiation. Eur J Biochem 234(3):843–848
25. van Belzen N, Dinjens WN, Eussen BH, Bosman FT (1998) Expression of differentiation-related genes in colorectal cancer: possible implications for prognosis. Histol Histopathol 13(4):1233–1242
26. Handa Y, Hikawa Y, Tochio N, Kogure H, Inoue M, Koshiba S, Guntert P, Inoue Y, Kigawa T, Yokoyama S, Nameki N (2010) Solution structure of the catalytic domain of the mitochondrial protein ICT1 that is essential for cell vitality. J Mol Biol 404(2):260–273. doi:10.1016/j.jmb.2010.09.033
27. Richter R, Rorbach J, Pajak A, Smith PM, Wessels HJ, Huynen MA, Smeitink JA, Lightowlers RN, Chrzanowska-Lightowlers ZM (2010) A functional peptidyl-tRNA hydrolase, ICT1, has been recruited into the human mitochondrial ribosome. EMBO J 29(6):1116–1125. doi:10.1038/emboj.2010.14
28. Bachmann IM, Halvorsen OJ, Collett K, Stefansson IM, Straume O, Haukaas SA, Salvesen HB, Otte AP, Akslen LA (2006) EZH2 expression is associated with high proliferation rate and aggressive tumor subgroups in cutaneous melanoma and cancers of the endometrium, prostate, and breast. J Clin Oncol 24(2):268–273
29. Kleer CG, Cao Q, Varambally S, Shen R, Ota I, Tomlins SA, Ghosh D, Sewalt RG, Otte AP, Hayes DF (2003) EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. Proc Natl Acad Sci U S A 100(20):11606–11611
30. Weikert S, Christoph F, Köllermann J, Müller M, Schrader M, Miller K, Krause H (2005) Expression levels of the EZH2 polycomb transcriptional repressor correlate with aggressiveness and invasive potential of bladder carcinomas. Int J Mol Med 16(2):349
31. Cao W, Ribeiro Rde O, Liu D, Saintigny P, Xia R, Xue Y, Lin R, Mao L, Ren H (2012) EZH2 promotes malignant behaviors via cell cycle dysregulation and its mRNA level associates with prognosis of patient with non-small cell lung cancer. PLoS One 7(12):e52984
32. Lin YW, Ren LL, Xiong H, Du W, Yu YN, Sun TT, Weng YR, Wang ZH, Wang JL, Wang YC, Cui Y, Sun DF, Han ZG, Shen N, Zou WP, Xu J, Chen HY, Cao WB, Hong J, Fang JY (2013) Role of STAT3 and Vitamin D Receptor in EZH2-mediated invasion of human colorectal cancer. J Pathol. doi:10.1002/path.4179
33. Zhang J-G, Guo J-F, Liu D-L, Liu Q, Wang J-J (2011) MicroRNA-101 exerts tumor-suppressive functions in non-small cell lung cancer through directly targeting enhancer of zeste homolog 2. J Thorac Oncol 6(4):671–678