Genetics and Molecular Research

Selecting key genes associated with osteosarcoma based on a differential expression network

Y.B. Wang[1], N. Jia[1], C.M. Xu[2], L. Zhao[1], Y. Zhao[1], X. Wang[1] and T.H. Jia[3]

[1]Department of Hand Surgery, The Third Hospital of Jinan, Jinan, China
[2]Department of Infectious Disease, The Third Hospital of Jinan, Jinan, Shandong, China
[3]Department of Osteology, Jinan Central Hospital Affiliated to Shandong University, Jinan, China

Correspondence author: T.H. Jia
E-mail: tanghong_jia@yeah.net

Genet. Mol. Res. 14 (4): 17708-17717 (2015)
Received August 17, 2015
Accepted October 8, 2015
Published December 21, 2015
DOI http://dx.doi.org/10.4238/2015.December.21.44

ABSTRACT. Despite recent advances in osteosarcoma diagnosis and therapy, much remains unclear about the molecular mechanisms involved in the disorder, and the discovery of novel drug-targeted genes is essential. We explored the potential molecular mechanisms and target genes involved in the development and progression of osteosarcoma. First, we identified the differentially expressed genes in osteosarcoma patients and matching normal controls. We then constructed a differential expression network based on differential and non-differential interactions. Pathway-enrichment analysis was performed based on the nodes contained in the main differential expression network. Centrality analysis was used to select hub genes that may play vital roles in the progression of human osteosarcoma. Our research revealed a total of 176 differentially expressed genes including 82 upregulated and 94 downregulated genes. A differential expression network was constructed that included 992 gene pairs (1043 nodes). Pathway-enrichment analysis indicated that the nodes in the

differential expression network were mainly enriched in several pathways such as those involved in cancer, cell cycle, ubiquitin-mediated proteolysis, DNA replication, ribosomes, T-cell receptor signaling, spliceosomes, neurotrophin signaling, oxidative phosphorylation, and tight junctions. Six hub genes (*APP*, *UBC*, *CAND1*, *RPA*, *YWHAG*, and *NEDD8*) were discovered; of these, two genes (*UBC* and *RPA*) were also found to be disease genes. Our study predicted that *UBC* and *RPA* had potential as target genes for the diagnosis and treatment of osteosarcoma.

Key words: Osteosarcoma; Differential expression network; Centrality analysis; Pathway-enrichment analysis

INTRODUCTION

Osteosarcoma (OSA) is the most frequently diagnosed primary bone tumor, and mainly occurs in children and young adults, with a second peak in middle age (Osaki et al., 2011). OSA is characterized by aberrant proliferation of malignant mesenchymal cells accompanied by the production of an osteoid matrix, immature bone, and intricate karyotypes (Federman et al., 2009). The tumor is characterized by very high morbidity, early location metastasis, and poor survival rate (Nagarajan et al., 2011; Salah et al., 2014). Currently, the prognosis for patients with metastatic diseases including OSA remains serious. Although many studies have described significant advances in surgery and adjuvant chemotherapy in OSA (Bacci et al., 2006; D'Adamo, 2011), surgery cannot halt the rapid metastasis of the tumor lesions, and chemotherapy is largely restricted by the development of resistance and severe side effects. Therefore, the discovery of new targets that have potential for OSA diagnosis and therapy is imperative.

Gene copy number alterations in OSA have been confirmed by comparative genomic hybridization (CGH) and single-nucleotide polymorphism (SNP) microarray analysis following the introduction of high-speed and high-throughput DNA microarray technologies. It is universally acknowledged that the occurrence and development of OSA is closely associated with the chromosome arms 6p, 8q, and 17p, and there are many additional reported regions (Squire et al., 2003; Kresse et al., 2010). The effects of copy number changes can be demonstrated by the upregulation or downregulation of gene expression levels in the affected chromosomal regions. To date, various published works have focused on analyzing OSA-related gene expression individually, but few researchers have explored the underlying mechanisms and target genes of OSA using robust bioinformatics.

Recently, a "differential network" (DN) technique (Ideker and Krogan, 2012) has been developed to extract disease-related edges by comparing interactions occurring across different static networks. Since genes and gene products operate not as a single unit but as part of a biochemical interaction, we assume that molecular interactions are disrupted by epigenetic or genetic factors; the disruption ultimately leads to molecular dysfunction. Therefore, the "dysfunctional interaction" concept, defined by the molecular interactions that show obvious differences between the wild-type and the disease condition, is proposed as a means of modeling the underlying human disease development and progression. However, a molecular interaction comprises two nodes and one edge, and the quantitative assessment of its change under different conditions is not straightforward. We combine the two types of perturbations when referring to

"dysfunctional interaction". Type I dysfunctional interactions are caused by edge perturbations. In other words, the co-expression relationship of the molecular interaction is changed differentially, and it is called a "differential interaction" because it directly changes the molecular interactions. Type II dysfunctional interactions are called "non-differential interactions" because they indirectly affect interactions through node changes. Note that non-differential interactions involve an edge that lacks significant differential strength, but its two linked nodes are both differentially expressed genes, and are therefore closely associated with biological processes. With the dysfunctional interaction concept, a novel type of molecular network, the "differential expression network" (DEN), has been developed. Compared with the traditional differential gene (DG) and DN techniques, DEN is better at describing the phenotype differences at the network level. From the network perspective, DEN not only includes DG and DN, but also covers "non-differential interactions", which are all missed by the DN method.

In this study, we constructed a DEN by extracting "differential interactions" and "non-differential interactions", which are capable of characterizing the initiation and progression of OSA. First, we downloaded the microarray data of the OSA model and sham controls from the ArrayExpress database. The altered expression profile was analyzed to identify the differentially expressed genes (DEGs). Additionally, the DEN was built based on the protein-protein interactions (PPI) combined with the gene expression profiles to determine hub genes via the degree centrality analysis of the DEN. Furthermore, we employed Database for Annotation, Visualization and Integrated Discovery (DAVID) software to show the significant Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways included in the DEN revealing abnormal biological processes. In our study, the elucidation of genes and pathways using the DEN method revealed potential new therapeutic targets.

MATERIAL AND METHODS

Data source

We extracted the gene microarray expression profiles of osteoblasts and mesenchymal stem cells (MSCs) E-GEOD-33382 (Kresse et al., 2010) from the ArrayExpress database (http://www.ebi.ac.uk/arrayexpress/). Gene expression analysis was performed on the seven normal controls and pretreatment diagnostic biopsies of 84 resectable osteosarcoma samples (Kresse et al., 2010). The clinical details of these samples are listed in Table 1. All the microarray datasets in CEL form and annotation information were downloaded for further exploration.

Screening of DEGs

After obtaining the raw expression datasets, quartile data normalization was performed using the Robust Multi-array Average (RMA) method (Ma et al., 2006). We then used the Student *t*-test to identify DEGs between OSA patients and normal controls (Ritchie et al., 2007). Finally, we selected the DEGs with values meeting the cut-off criteria (P value of less than 0.05 and |log-fold change (FC)| larger than 2).

Generic PPI network

Because proteins rarely exert their functions individually, it is very important to explore

protein interactions by studying functional protein groups (Srihari and Leong, 2012). For this paper, we downloaded the whole PPI datasets from the Biological General Repository for Interaction Datasets (BioGrid, http://thebiogrid.org/). Cytoscape 2.1 software was employed to establish PPI networks (Shannon et al., 2003). In the original PPI network, a total of 15,750 genes and 248,584 interactions were contained in the BioGrid. Based on the transcript data of E-GEOD-33382, we then secured another PPI network including 10,084 genes and 11,6546 interactions.

Table 1. Clinical details of the 84 patients selected.

Category	Patient characteristics	Number of biopsies (%)
Institution	LUMC, Netherlands	36 (42.9)
	WWUM, Germany	32 (38.1)
	IOR, Italy	12 (14.3)
	Sweden	3 (3.6)
	Norway	1 (1.2)
Gender	Male	54 (64.3)
	Female	29 (34.5)
	Unknown	1 (1.2)
Age	>20 years	19 (22.6)
	<20 years	64 (76.2)
	Unknown	1 (1.2)
Location	Femur	40 (47.6)
	Tibia/Fibula	28 (33.3)
	Humerus	11 (13.1)
	Axial skeleton	1 (1.2)
	Elsewhere	4 (4.8)
Histological subtype	Osteoblastic	52 (61.9)
	Minor subtype	11 (13.1)
	Chondroblastic	9 (10.7)
	Fibroblastic	7 (8.3)
	Telangiectatic	4 (4.8)
	Unknown	1 (1.2)
Metastasis	Yes	14 (16.7)
	No	69 (82.1)
Huvos grade	1 or 2	38 (45.2)
	3 or 4	33 (39.3)
	Unknown	14 (16.7)

LUMC = Leiden University Medical Center; WWUM = Westfälische Wilhelms-Universität Münster; IOR = Istituto Ortopedico Rizzoli.

Calculating Spearman correlation coefficients of gene pairs

The Spearman correlation coefficient method was applied to evaluate the strength of gene interactions. For each edge in the PPI network, the Spearman correlation coefficient was calculated separately using gene expression values under different conditions (healthy controls and OSA patients), which served as A1 and A2, respectively.

Determining the threshold of differential interaction

Two models (one for cases, the other for controls) were established randomly, and each model contained 200,000 gene pairs. The Spearman correlation coefficients in the two models (A1, A2) were computed. The absolute correlation coefficient values (|A1-A2|) were obtained. After aligning the absolute correlation coefficient values in descending order and setting the P value threshold at 0.01, we observed that the absolute value was 1.422. The Spearman value of the

116,546 relationships based on BioGrid and transcript data were arrayed in descending order. We selected those differential relationships that had an absolute correlation coefficient value of greater than 1.422, and at least one Spearman correlation coefficient of greater than 0.7.

Construction of DEN

The edges with absolute correlation coefficient values of greater than 1.422 and at least one of A1 or A2 greater than 0.7 were considered to represent differential interactions. Other gene pairs with |A1-A2| values of less than or equal to 1.422 and two nodes that were both DEGs were taken to represent non-differential interactions. The DEN was constructed by incorporating all the differential and non-differential interactions. The network was constructed using Cytoscape 2.1 software.

Node centrality analysis

We used classical network centrality analysis in this study. Network centrality analysis, in which each centrality presents a possible biological meaning in a protein network, plays a significant role in the investigation of biological networks. The centrality method involves degree centrality, closeness centrality, betweenness centrality, etc. As the simplest topological index, the degree corresponds to the number of adjacent nodes, where “adjacent” means directly connected (Koschützki and Schreiber, 2008). Nodes with a high degree are called “hub genes”, and combine some nodes with a lower degree, thereby indicating a central role in the network (He and Zhang, 2006). Networks that display a distribution approximating a power law are referred to as scale-free networks (Pietsch, 2006). A scale-free network is typically a natural network, is mainly dominated by hub genes, and is intrinsically robust to random attacks, but is susceptible to selected alterations (Jeong et al., 2001).

Identification of the disease genes contained in the DEN

We downloaded the genes associated with OSA (denoted as “disease genes” for convenience in this study) from the GeneCards database at the website http://www.genecards.org/. GeneCards is a recognized and comprehensive human gene database that provides all known and predicted transcriptomic, genomic, proteomic, and functional genetic information (Harel et al., 2009). The disease genes underlying disease development and progression in the DEN were selected in a complex statistical way.

Pathway-enrichment analysis

The KEGG pathway database is a comprehensive and authoritative database that provides almost all biochemical pathways. In our study, the online DAVID software, which includes a set of novel and powerful tools, was used for KEGG pathway enrichment to identify the main biochemical pathways for the nodes in DEN (Huang et al., 2007). We selected P values of less than 0.01 and gene counts of larger than or equal to 9 as the cut-off criteria for KEGG pathway-enrichment analysis.

RESULTS

Identification of DEGs

Based on the statistical analysis of microarray datasets between OSA patients and the normal controls, a total of 176 DEGs (82 upregulated and 94 downregulated genes) were screened according to the criteria: |logFC| > 2.0 and P value <0.05.

Construction of DEN

As described above, the DEN should be constructed by complementarily considering both differential and non-differential interactions. We identified 966 differential interactions with |A1-A2| >1.422 and at least one of A1 or A2 >0.7. Moreover, there were 26 gene pairs with |A1-A2| values of less than or equal to 1.422 but with corresponding nodes that were both DEGs. In other words, there were 26 non-differential interactions. Therefore, a DEN including 992 gene pairs (1043 nodes) was constructed. The main DEN is shown in Figure 1. By comprehensive statistical analysis, we constructed a DEN comprising 101 disease genes constituting 247 disease gene pairs.

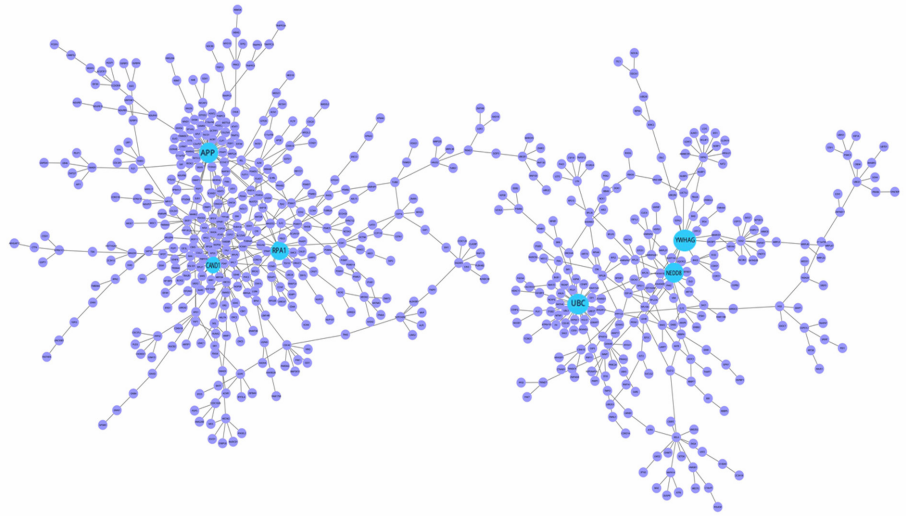

Figure 1. Differential expression network analysis of osteosarcoma. Nodes represent the proteins (genes) and edges represent the protein interaction. Blue indicates genes with a lower degree and green indicates hub genes.

Centrality analysis to obtain hub genes

Analysis of the nodes degree of the DEN revealed that the degree distribution was close to a power law, illustrating that the DEN was a scale-free network, as depicted in Figure 2. Six genes (*APP*, *UBC*, *CAND1*, *RPA*, *YWHAG*, and *NEDD8*) with high connectivity degree (>14) were selected as the hub nodes and might play an important role in the progression of OSA. The connectivity degrees of the hub genes are shown in Table 2. Two of the hub genes, *UBC* and *RPA*, are also disease genes, which indicates their more crucial role in the oncogenesis of OSA.

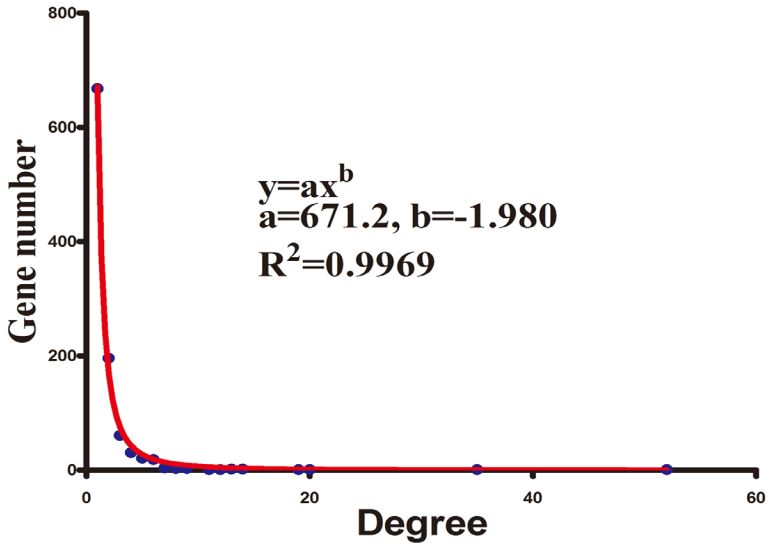

Figure 2. Scattergram of gene degree distribution in the differential expression network. The degree distribution presents a power law, indicating the character of the scale-free network.

Table 2. Statistical results of connectivity degrees and other information of hub genes.

Gene	Gene description	Degree	DEGs	Disease genes
APP	Amyloid precursor protein	52	False	False
UBC	Ubiquitin-conjugating enzyme gene	35	False	True
CAND1	Cullin-associated and neddylation-dissociated 1	20	False	False
RPA	Replication protein A	19	False	True
YWHAG	Musculus tyrosine 3 monooxygenase/tryptophan 5-monooxygenase activation protein	14	False	False
NEDD8	Neural precursor cell expressed, developmentally down-regulated 8	14	False	False

DEGs = differentially expressed genes. Gene symbols also represent the corresponding proteins; degree represents the degree of connectivity for each gene.

KEGG pathway-enrichment analysis

To gain further insight into the function of the nodes in the DEN, the DAVID software was used to determine the significant dysregulated KEGG enrichment pathways. The obtained pathways with P values < 0.01 and gene counts ≥9 are shown in Table 3. From the results, we can see that the genes in the DEN were mainly enriched in pathways involving cancer, the cell cycle, ubiquitin-mediated proteolysis, DNA replication, ribosomes, T-cell receptor signaling, spliceosomes, neurotrophin signaling, oxidative phosphorylation, and tight junctions.

DISCUSSION

OSA is the most common malignant bone cancer that occurs in humans. Although the aggressive use of surgical techniques and chemotherapy have improved survival, the prognosis for OSA patients remains poor, and treatment still presents severe challenges (Akiyama et al., 2008). Therefore, more effective anti-OSA targets are urgently sought.

Table 3. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway-enrichment results.

ID	Term	Count	P value
hsa03010	Ribosome	30	8.41E-12
hsa04120	Ubiquitin-mediated proteolysis	34	4.60E-09
hsa04110	Cell cycle	29	3.67E-07
hsa03420	Nucleotide excision repair	14	2.45E-05
hsa05130	Pathogenic *Escherichia coli* infection	16	2.71E-05
hsa03050	Proteasome	14	5.30E-05
hsa05120	Epithelial cell signaling in *pylori* infection	16	2.40E-04
hsa03430	Mismatch repair	9	2.68E-04
hsa04114	Oocyte meiosis	21	3.85E-04
hsa05220	Chronic myeloid leukemia	16	7.32E-04
hsa04660	T-cell receptor signaling pathway	20	8.19E-04
hsa05212	Pancreatic cancer	15	1.46E-03
hsa03040	Spliceosome	21	2.24E-03
hsa04722	Neurotrophin-signaling pathway	20	4.29E-03
hsa05215	Prostate cancer	16	4.34E-03
hsa03030	DNA replication	9	6.55E-03
hsa00190	Oxidative phosphorylation	20	7.21E-03
hsa05200	Pathways in cancer	40	7.39E-03
hsa04914	Progesterone-mediated oocyte maturation	15	7.88E-03
hsa04530	Tight junction	20	9.94E-03

In this study, we researched the gene expression profiles of OSA through DEN, expecting to select target genes associated with OSA. Analysis of the constructed DEN revealed that six genes (*APP*, *UBC*, *CAND1*, *RPA*, *YWHAG*, and *NEDD8*) were hub nodes and none was a DEG. DEGs, showing significantly different expression profiles in the case and control groups, are usually found using traditional statistical techniques, such as the *t*-test or fold change (Goñi et al., 2008; Ray and Zhang, 2010). Although DEGs are regarded as candidates for a role in pathogenesis, they are generally selected out separately, while co-expression of genes is ignored (Kostka and Spang, 2004). In this paper, six hub genes, not including the DEGs, were screened by DEN, which comprised “differential interactions” and “non-differential interactions”. DEN, which complements traditional methods, can be used to interpret gene interactions from a new perspective. Among the six hub genes obtained using DEN, *UBC* and *RPA* were also disease genes, which suggested that *UBC* and *RPA* might be more selectively involved in OSA.

By performing KEGG pathway enrichment analysis of the nodes contained in DEN, we found that the hub gene *UBC* participates in the ubiquitin-mediated proteolysis pathway. The expression of ubiquitin-conjugating enzyme (E2) UBC13, which belongs to the UBC family, is significantly upregulated in metastatic breast cancer (Fidler, 2003). UBC13 first heterodimerizes with Uev1a and then catalyzes the formation of lysine 63-linked polyubiquitin chains, which govern the PPIs associated with protein kinase activation and DNA damage repair (Bhoj and Chen, 2009; Wang et al., 2012). For some immune cells, UBC13 is indispensable for IκB kinase (IKK)-NF-κB activation, but a more important function of UBC13 is the activation of the MAPK signaling pathway (Yamamoto et al., 2006a; Yamamoto et al., 2006b). It has been reported that UBC13 is also required for triggering MEKK1 (mitogen-activated protein kinase kinase kinase 1), TGFβ (transforming growth factor β)-activating kinase 1 (TAK1), and the downstream MAPK cascade (Matsuzawa et al., 2008). Crucially, MEKK1 and TAK1 are also required for the metastasis of cancer cells (Cuevas et al., 2006; Safina et al., 2008). A recently published paper showed that UBC13 controls breast cancer metastasis via TAK1-p38 MAP kinase cascades (Wu et al., 2014).

The KEGG functional enrichment results showed that RPA is involved in the DNA replication pathway. RPA is a heterotrimeric ssDNA-binding protein and plays a crucial role in S-phase DNA

replication, DNA excision repair, DNA recombination, and other DNA metabolic pathways (Wold, 1997; Fanning et al., 2006). Additionally, RPA is involved in the ATR/Chk1 pathway and it recruits and interacts with other proteins, such as ATRIP, Nbs1, Rad17, and Rad9, that are required for the replication stress response (Zou et al., 2003; Majka et al., 2006; Xu et al., 2008; Oakley et al., 2009). Recruitment of ATRIP, Nbs1, Rad17, and Rad9 entails the loading of TopBP1 followed by the activation of ATR (Zou and Elledge, 2003; Xu et al., 2008; Shiotani et al., 2013). This triggers a critical surveillance network of signaling pathways that mediate the cellular response to replication stress. Cancer cells with activated oncogenes cause a massive increase in stress during replication. Compared with normal cells, the presence of higher replication stress levels in cancer cells opens up a therapeutic opportunity to target RPA for preferentially killing cancer cells.

CONCLUSION

In light of the preliminary study, we discovered that the pathogenesis of osteosarcoma is closely related to several other pathways including those involving cancer, the cell cycle, ubiquitin-mediated proteolysis, DNA replication, and ribosomes. Based on the DEN, we also confirmed that six genes (*APP*, *UBC*, *CAND1*, *RPA*, *YWHAG*, and *NEDD8*) might play key roles in osteosarcoma, and that two genes (*UBC* and *RPA*) have potential as biomarkers for the diagnosis and treatment of human osteosarcoma.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank Wen Sun for useful suggestions regarding this study. We also thank all the members of the Ji'nan Evidence-Based Medicine Science-Technology Center for helpful advice on statistics and computation.

REFERENCES

Akiyama T, Dass CR and Choong PF (2008). Novel therapeutic strategy for osteosarcoma targeting osteoclast differentiation, bone-resorbing activity, and apoptosis pathway. *Mol. Cancer Ther.* 7: 3461-3469.

Bacci G, Longhi A, Ferrari S, Mercuri M, et al. (2006). Prognostic factors in non-metastatic Ewing's sarcoma tumor of bone: an analysis of 579 patients treated at a single institution with adjuvant or neoadjuvant chemotherapy between 1972 and 1998. *Acta Oncol.* 45: 469-475.

Bhoj VG and Chen ZJ (2009). Ubiquitylation in innate and adaptive immunity. *Nature* 458: 430-437.

Cuevas BD, Winter-Vann AM, Johnson NL and Johnson GL (2006). MEKK1 controls matrix degradation and tumor cell dissemination during metastasis of polyoma middle-T driven mammary cancer. *Oncogene* 25: 4998-5010.

D'Adamo DR (2011). Appraising the current role of chemotherapy for the treatment of sarcoma. *Semin. Oncol.* 38: S19-S29.

Fanning E, Klimovich V and Nager AR (2006). A dynamic model for replication protein A (RPA) function in DNA processing pathways. *Nucleic Acids Res.* 34: 4126-4137.

Federman N, Bernthal N, Eilber FC and Tap WD (2009). The multidisciplinary management of osteosarcoma. *Curr. Treat. Options Oncol.* 10: 82-93.

Fidler IJ (2003). The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. *Nat. Rev. Cancer* 3: 453-458.

Goñi J, Esteban FJ, de Mendizábal NV, Sepulcre J, et al. (2008). A computational analysis of protein-protein interaction networks in neurodegenerative diseases. *BMC Syst. Biol.* 2: 52.

Harel A, Inger A, Stelzer G, Strichman-Almashanu L, et al. (2009). GIFtS: annotation landscape analysis with GeneCards. *BMC*

Bioinformatics 10: 348.
He X and Zhang J (2006). Why do hubs tend to be essential in protein networks? *PLoS Genet.* 2: e88.
Huang DW, Sherman BT, Tan Q, Collins JR, et al. (2007). The DAVID Gene Functional Classification Tool: a novel biological module-centric algorithm to functionally analyze large gene lists. *Genome Biol.* 8: R183.
Ideker T and Krogan NJ (2012). Differential network biology. *Mol. Syst. Biol.* 8: 565.
Jeong H, Mason SP, Barabási AL and Oltvai ZN (2001). Lethality and centrality in protein networks. *Nature* 411: 41-42.
Koschützki D and Schreiber F (2008). Centrality analysis methods for biological networks and their application to gene regulatory networks. *Gene Regul. Syst. Biol.* 2: 193-201.
Kostka D and Spang R (2004). Finding disease specific alterations in the co-expression of genes. *Bioinformatics* 20: i194-i199.
Kresse SH, Szuhai K, Barragan-Polania AH, Rydbeck H, et al. (2010). Evaluation of high-resolution microarray platforms for genomic profiling of bone tumours. *BMC Res. Notes* 3: 223.
Ma L, Robinson LN and Towle HC (2006). ChREBP*Mlx is the principal mediator of glucose-induced gene expression in the liver. *J. Biol. Chem.* 281: 28721-28730.
Majka J, Binz SK, Wold MS and Burgers PM (2006). Replication protein A directs loading of the DNA damage checkpoint clamp to 5'-DNA junctions. *J. Biol. Chem.* 281: 27855-27861.
Matsuzawa A, Tseng PH, Vallabhapurapu S, Luo JL, et al. (2008). Essential cytoplasmic translocation of a cytokine receptor-assembled signaling complex. *Science* 321: 663-668.
Nagarajan R, Kamruzzaman A, Ness KK, Marchese VG, et al. (2011). Twenty years of follow-up of survivors of childhood osteosarcoma: a report from the Childhood Cancer Survivor Study. *Cancer* 117: 625-634.
Oakley GG, Tillison K, Opiyo SA, Glanzer JG, et al. (2009). Physical interaction between replication protein A (RPA) and MRN: involvement of RPA2 phosphorylation and the N-terminus of RPA1. *Biochemistry* 48: 7473-7481.
Osaki M, Takeshita F, Sugimoto Y, Kosaka N, et al. (2011). MicroRNA-143 regulates human osteosarcoma metastasis by regulating matrix metalloprotease-13 expression. *Mol. Ther.* 19: 1123-1130.
Pietsch W (2006). Derivation of the percolation threshold for the network model of Barabási and Albert. *Phys. Rev. E. Stat. Nonlin. Soft Matter Phys.* 73: 066112.
Ray M and Zhang W (2010). Analysis of Alzheimer's disease severity across brain regions by topological analysis of gene co-expression networks. *BMC Syst. Biol.* 4: 136.
Ritchie ME, Silver J, Oshlack A, Holmes M, et al. (2007). A comparison of background correction methods for two-colour microarrays. *Bioinformatics* 23: 2700-2707.
Safina A, Ren MQ, Vandette E and Bakin AV (2008). TAK1 is required for TGF-beta 1-mediated regulation of matrix metalloproteinase-9 and metastasis. *Oncogene* 27: 1198-1207.
Salah S, Ahmad R, Sultan I, Yaser S, et al. (2014). Osteosarcoma with metastasis at initial diagnosis: Current outcomes and prognostic factors in the context of a comprehensive cancer center. *Mol. Clin. Oncol.* 2: 811-816.
Shannon P, Markiel A, Ozier O, Baliga NS, et al. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* 13: 2498-2504.
Shiotani B, Nguyen HD, Håkansson P, Maréchal A, et al. (2013). Two distinct modes of ATR activation orchestrated by Rad17 and Nbs1. *Cell Rep.* 3: 1651-1662.
Squire JA, Pei J, Marrano P, Beheshti B, et al. (2003). High-resolution mapping of amplifications and deletions in pediatric osteosarcoma by use of CGH analysis of cDNA microarrays. *Genes Chromosomes Cancer* 38: 215-225.
Srihari S and Leong HW (2012). Temporal dynamics of protein complexes in PPI networks: a case study using yeast cell cycle dynamics. *BMC Bioinformatics* 13: S16.
Wang G, Gao Y, Li L, Jin G, et al. (2012). K63-linked ubiquitination in kinase activation and cancer. *Front. Oncol.* 2: 5.
Wold MS (1997). Replication protein A: a heterotrimeric, single-stranded DNA-binding protein required for eukaryotic DNA metabolism. *Annu. Rev. Biochem.* 66: 61-92.
Wu X, Zhang W, Font-Burgada J, Palmer T, et al. (2014). Ubiquitin-conjugating enzyme Ubc13 controls breast cancer metastasis through a TAK1-p38 MAP kinase cascade. *Proc. Natl. Acad. Sci. U. S. A.* 111: 13870-13875.
Xu X, Vaithiyalingam S, Glick GG, Mordes DA, et al. (2008). The basic cleft of RPA70N binds multiple checkpoint proteins, including RAD9, to regulate ATR signaling. *Mol. Cell Biol.* 28: 7345-7353.
Yamamoto M, Okamoto T, Takeda K, Sato S, et al. (2006a). Key function for the Ubc13 E2 ubiquitin-conjugating enzyme in immune receptor signaling. *Nat. Immunol.* 7: 962-970.
Yamamoto M, Sato S, Saitoh T, Sakurai H, et al. (2006b). Cutting Edge: Pivotal function of Ubc13 in thymocyte TCR signaling. *J. Immunol.* 177: 7520-7524.
Zou L and Elledge SJ (2003). Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science* 300: 1542-1548.
Zou L, Liu D and Elledge SJ (2003). Replication protein A-mediated recruitment and activation of Rad17 complexes. *Proc. Natl. Acad. Sci. U. S. A.* 100: 13827-13832.