Establishing an osteosarcoma associated protein-protein interaction network to explore the pathogenesis of osteosarcoma

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Abstract

Background: The aim of this study was to establish an osteosarcoma (OS) associated protein-protein interaction network and explore the pathogenesis of osteosarcoma.

Methods: The gene expression profile GSE9508 was downloaded from the Gene Expression Omnibus database, including five samples of non-malignant bone (the control), seven samples for non-metastatic patients (six of which were analyzed in duplicate), and 11 samples for metastatic patients (10 of which were analyzed in duplicate). Differentially expressed genes (DEGs) between osteosarcoma and control samples were identified by packages in R with the threshold of |logFC (fold change)| > 1 and false discovery rate < 0.05. Osprey software was used to construct the interaction network of DEGs, and genes at protein-protein interaction (PPI) nodes with high degrees were identified. The Database for Annotation, Visualization and Integrated Discovery and WebGestalt software were then used to perform functional annotation and pathway enrichment analyses for PPI networks, in which \( P < 0.05 \) was considered statistically significant.

Results: Compared to the control samples, the expressions of 42 and 341 genes were altered in non-metastatic OS and metastatic OS samples, respectively. A total of 15 significantly enriched functions were obtained with Gene Ontology analysis \( (P < 0.05) \). The DEGs were classified and significantly enriched in three pathways, including the tricarboxylic acid cycle, lysosome and axon guidance. Genes such as HRAS, IDH3A, ATP6ap1, ATP6V0D2, SEMA3F and SEMA3A were involved in the enriched pathways.

Conclusions: The hub genes from metastatic OS samples are not only bio-markers of OS, but also help to improve therapies for OS.

Keywords: Osteosarcoma, Differentially expressed genes, Protein-protein interaction network, Pathway enrichment analysis

Background

Osteosarcoma (OS), an aggressive malignant neoplasm, is the most common primary bone cancer in children and adolescents [1]. Generally, OS occurs in the long bones of the extremities, such as the femur, the tibia and the humerus [2]. Previous studies have demonstrated that it is possible to cure more than 60% of non-metastatic OS with neoadjuvant chemotherapy [3]. However, the 5-year survival rate of patients with metastases at diagnosis is less than 30% [4,5]. Although the mortality has been decreased by about 1.3% per year as a result of improvement in surgical techniques and chemotherapy, there is no reliable means of predicting outcomes at the time of presentation. Thus, identifying potential therapeutic targets is very important in the treatment of patients with metastatic OS.

OS is a heterogeneous disease including metastatic and non-metastatic complex etiologies. Extensive efforts have been made to explore the underlying pathogenesis and molecular mechanisms. As reported [6], Ezrin links the plasma membrane to the actin cytoskeleton where it plays a pivotal role in the metastatic progression of cancers. Ezrin immunoreactivity can be detected in the majority of
patients with non-metastatic OS of the extremity [6]. However, metastatic cells with an efficiently regulated actin cytoskeleton may have a greater fluency and flexibility in managing energetic needs during times of stress than non-metastatic cells. The dysregulation of Ezrin may lead to an urgent but failed attempt by cells to correct this metabolic defect [7]. Therefore, Ezrin is higher expressed in metastatic cells. There are also many other genes that are changed in metastatic OS. Clusterin, collagen 4A3, integrin β4, integrin β2, vascular endothelial growth factor, connective tissue growth factor, integrin-α5, cadherin 2, cxc ligand 12, cxc chemokiner receptor 4, cell division cycle 5 like, and microRNA215 are found to be upregulated in metastatic OS, while cadherin 11, MME, KIAA1959 and S100A6 are downregulated in metastatic OS [8-11]. Furthermore, some prognosis factors have been demonstrated as potential therapeutic targets, such as reversion-inducing-cysteine-rich protein with kazal motifs and urokinase plasminogen activator [10,11]. The receptor tyrosine kinase-like orphan receptor 2 has also been identified as being important in the development of metastatic OS [8,12]. In spite of the great progress in the pathogenesis of OS, the molecular mechanisms have not been fully elucidated.

In this paper, we focus on exploring the molecular mechanisms of OS with a computational bioinformatics analysis of gene expression. We anticipate that candidate genes identified by our method may provide a new therapeutic approach for OS.

Methods

Affymetrix microarray

The gene profile of GSE9508 was downloaded from the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/), which was based on the platform of Agilent-Whole Human Genome Oligo Microarray G4112A, condensed. This expression dataset was deposited by Endo-Munoz [13]. Biopsies were collected at the time of diagnosis, before preoperative chemotherapy, near the tumor-bone interface and were representative of the tumor. A total of 39 samples were derived from 22 patients (11 males and 11 females), including five samples of non-malignant bone (the control), seven samples for non-metastatic patients (six of which were analyzed in duplicate), and 11 samples for metastatic patients (10 of which were analyzed in duplicate). Non-malignant bone was collected from patients presenting for hip or knee replacement surgery.

Data processing and identification of differential expression analysis

Firstly, data at the probe-level in CEL format were converted into expression measures and median normalization was performed by R language [14,15]. Then, differentially expressed genes (DEGs) between the samples were identified with the Limma package [16] in R. The Benjamini & Hochberg [17] method was used to adjust the raw P-values into the false discovery rate (FDR). |logFC| (fold change) > 1 and FDR < 0.05 were chosen as the cut-off criteria.

Construction of protein-protein interaction network

The interactions between the DEGs in the groups were analyzed and the protein-protein interaction (PPI) networks were constructed with Osprey using the data from the General Repository for Interaction Datasets [18,19] and Bio-molecular Interaction Network Database [20]. A total of 159 interaction pairs were analyzed.

Gene ontology enrichment analysis

Gene ontology (GO) analysis has been used frequently in functional studies of large-scale genomic or transcriptomic data [21,22]. GO provides three structured networks of defined terms, namely biological process, molecular function, and cellular compartment [23]. To functionally classify PPI nodes with a high degree (number of interactions), we performed biological process ontology. We performed GO enrichment analysis with The Database for Annotation, Visualization and Integrated Discovery (DAVID), which provides functional interpretation for researchers to study biological information behind large lists of genes [24]. A P-value less than 0.05 was considered statistically significant.

Pathway enrichment analysis

We utilized the WebGestalt web site (web-based gene set analysis toolkit, http://genereg.ornl.gov/webgestalt) [25,26] for pathway enrichment analysis. A P-value less than 0.05 was considered as the cut-off criterion.

Results

Differential gene expression in osteosarcoma samples

After data normalization (Figure 1), a total of 42 and 341 DEGs with the FDR < 0.05 and |logFC| > 1 were identified between non-metastatic or metastatic samples and the control. These findings indicated that genetic abnormalities in metastatic samples were significantly higher than in non-metastatic samples.

Protein-protein interaction network

Interaction networks of DEGs were constructed with Osprey (Figure 2A). As a result, interaction relationships of 145 DEGs were obtained. Several PPI nodes were high in degrees in the network, such as POLE (10), HRAS (9), H3F3, HSPA4, ADCY2 (8), IDH3A, ATP6ap1 and ATP6V0D2. All these nodes were obtained from metastatic samples. A high level of connectivity is shown in the network (Figure 2A). All of the five key hub proteins (POLE (10), HRAS (9), H3F3, HSPA4, ADCY2 (8)) and connections were then deleted. As shown in Figure 2B, integrity of the network was disrupted due to the absence
of key hubs and connections between the nodes. Compared to the previous network, isolated node clusters were increased in the new network.

**Gene ontology analysis**

We performed functional enrichment analysis using the online biological classification tool DAVID, and obtained 15 significantly enriched functions (Table 1, \( P < 0.05 \)). The PPI nodes of high degrees were classified with GO analysis according to their functions. The most remarkable function was regulation of adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains (GO:0002822, \( P = 0.01166 \)). The other significant functions included mRNA metabolic process (GO:0016071, \( P = 0.01427 \)), negative regulation of molecular function (GO:0044092, \( P = 0.02485 \)), nuclear-transcribed mRNA catabolic process (GO:0000184, \( P = 0.02712 \)) and so on. Genes such as C3, TBX21, HNRNPL, NCBP2, UPF3A, HRAS, SEMA3F and SEMA3A were involved in this GO analysis.

**Pathway enrichment analysis of the protein-protein interaction network**

A total of three significant pathways with \( P \)-value less than 0.05 were enriched (Table 2). The most significant pathway was the citrate cycle (hsa00020, tricarboxylic acid cycle (TCA) cycle) with a \( P \)-value of 0.006557. Three genes were involved in the TCA cycle, including SUCLA2, OGDH and IDH3A. The other two significant pathways were the lysosome (\( P = 0.007354 \)) and axon guidance (\( P = 0.009702 \)). Genes associated with the lysosome pathway were IDS, ATP6AP1, GALNS, ATP6V0D2 and AP3B1. Five genes were involved in axon guidance, including HRAS, RGS3, SEMA3F, SEMA3A, APHBI and EPHB1.

**Discussion**

In the present study, we investigated gene expression profile in OS patients and explored the potential molecular mechanisms using computational bioinformatics methods. Results showed that expressions of 42 and 341 genes were altered in non-metastatic OS and metastatic OS samples, respectively. The DEGs were significantly enriched in three pathways, including the TCA cycle, lysosome and axon guidance. PPI nodes such as HRAS, IDH3A, ATP6ap1 and ATP6V0D2 were of high degrees in the network, all of which were obtained from metastatic samples.

**IDH3A**, a mitochondrial NAD-dependent isocitrate dehydrogenase involved in the TCA cycle, was identified to exert an effect on OS in our study. OS cells have been
demonstrated to have an impaired TCA cycle and partially compensate for the mitochondrial defect [27]. This gene was differentially expressed in metastatic samples compared with the non-metastatic and non-malignant samples, suggesting that \textit{IDH3A} is closely related to migration of metastatic OS cells. In the present study, expression of oncogenic HRAS was increased in metastatic samples. This result was in accordance with a previous study where expression of oncogenic HRAS induced permanent growth arrest in human OS cell lines [28].

Several genes which are specifically found in metastatic OS cells are associated with inhibition of osteoclast differentiation in osteosarctic patients, including \textit{ATP6ap1} (Ac45), \textit{ATP6V0D2} (an isoform of V-ATPase subunit d) and \textit{SEMA}
ATP6ap1, an accessory subunit of vacuolar-type H+−ATPases (V-ATPases), is identified to be involved in the progress of OS formation. Significantly reduced normal osteoclast formation and inhibition of osteoclast differentiation are found in Ac45 knockdown leads to the decrease in the number of osteoclasts, which does not result from abnormal apoptosis but from decreased osteoclast precursor cell proliferation and fusion. This decrease is partially due to the downregulation of extracellular signal-regulated kinase phosphorylation and FBJ OS oncogene (c-fos), nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1), and Transmembrane 7 superfamily member 4 expression. Currently, ATP6ap1 is regarded as a novel therapeutic target for osteolytic disease [29].

ATP6v0d2 was demonstrated to downregulate the migration of metastatic OS cells in this study. Indeed, it has been identified to mediate osteoclast differentiation [30] and bone formation [31,32]. ATP6v0d2, involved in resorption of the bone extracellular matrix, is induced by receptor activator of NF-κB ligand (RANKL) which is necessary for osteoclast formation. Thus, ATP6v0d2, as well as NFAT-c1, TRAP, DC-STAMP and cathepsin K, is considered one of the osteoclast marker genes [33,34].

We also observed the differential expression of semaphoring family genes (SEMA3F, SEMA3A), a family of proteins that exert a profound impact on cancers [35]. SEMA3F binds to neuropilin-2 (NRP2) and acts as a tumor suppressor [36]. NRP2 has been reported to affect the increased vascularization and correlate with advanced tumor progression and prognosis in OS [35-38]. Poorer prognosis is related to OS with NRP2 rather than those without NRP2 [38]. In contrast, SEMA-3A binds to NRP1 which induces the collapse of neuronal growth cones. Furthermore, ATP6ap1 is regarded as a novel therapeutic target for osteolytic disease [29].

### Table 1 Functional enrichment of differentially expressed genes between metastatic samples, non-metastatic samples and non-malignant samples (P < 0.05)

| Category Term                                                                 | Count | P-value   | Genes                                             |
|-------------------------------------------------------------------------------|-------|-----------|---------------------------------------------------|
| GO:0002822 Regulation of adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains | 4     | 0.01166   | C3, TBX21, RARA, FOXP3                             |
| GO:0002819 Regulation of adaptive immune response                              | 4     | 0.01225   | C3, TBX21, RARA, FOXP3                             |
| GO:0016071 mRNA metabolic process                                             | 9     | 0.01427   | HNRNPL1, NCBP2, UFP3A, C17orf71, RNMT, GTF2F1, SYNCBP, SNRPC, SF3A1 |
| GO:0044092 Negative regulation of molecular function                          | 8     | 0.02485   | MDFI, KDMD1A, ADOV2, RGS3, WWP2, BUB1B, FOXP3, ANSPTL4 |
| GO:0000184 Nuclear-transcribed mRNA catabolic process, nonsense-mediated decay | 3     | 0.02712   | NCBP2, UFP3A, C17orf71                            |
| GO:007626 Locomotory behavior                                                 | 7     | 0.03055   | HR3, CCR3, SEMA3F, MYO15A, CHRNA4, SEMA3A, ESPN    |
| GO:0051129 Negative regulation of cellular component organization              | 5     | 0.03381   | SEMA3F, RDX, CLASP2, SEMA3A, FOXP3                 |
| GO:0048841 Regulation of axon extension involved in axon guidance             | 2     | 0.03386   | SEMA3F, SEMA3A                                     |
| GO:0048843 Negative regulation of axon extension involved in axon guidance    | 2     | 0.03386   | SEMA3F, SEMA3A                                     |
| GO:006370 mRNA capping                                                        | 2     | 0.03386   | NCBP2, RNMT                                       |
| GO:0000956 Nuclear-transcribed mRNA catabolic process                         | 3     | 0.03609   | NCBP2, UFP3A, C17orf71                            |
| GO:0045621 Positive regulation of lymphocyte differentiation                  | 3     | 0.03609   | RARA, INPP5D, AP3B1                                |
| GO:0051249 Regulation of lymphocyte activation                                 | 5     | 0.03845   | TBX21, RARA, INPP5D, FOXP3, AP3B1                 |
| GO:0009452 RNA capping                                                        | 2     | 0.04215   | NCBP2, RNMT                                       |
| GO:0050919 Negative chemotaxis                                                | 2     | 0.04215   | SEMA3F, SEMA3A                                     |

A total of three significant pathways with P < 0.05 were enriched. TCA, tricarboxylic acid cycle.

### Table 2 Pathway enrichment in the protein-protein interaction network using the WebGestalt

| Category Term | Count | P-value | Differentially expressed genes |
|---------------|-------|---------|--------------------------------|
| hsa00020: Citrate cycle (TCA cycle) | 3     | 0.00657 | SUCL2, OGDH, IDH3A             |
| hsa04142: Lysosome | 5     | 0.00735 | IDS, ATP6AP1, GALNS, ATP6V0D2, AP3B1 |
| hsa04360: Axon guidance | 5     | 0.00970 | HRAS, RGS3, SEMA3F, SEMA3A, Ephb1 |
inhibition of migration that shows the major differences between metastatic and non-metastatic OS cells [13]. Although these PPI nodes were isolated and suspected to be involved in the mechanism of metastatic OS, many of them are not shown to participate in this process in experimental research. Further research will be needed to investigate the role played by the genes present in the mechanism of metastatic OS.

Conclusions
We have demonstrated 42 and 341 DEGs in non-metastatic and metastatic OS samples compared to the control samples. Significantly enriched pathways including the TCA cycle, lysosome and axon guidance are dysfunctional in metastatic OS cells. However, the specific markers at the molecular level in the pathogenesis of OS are not fully understood. More studies are warranted to further explore the molecular mechanisms of OS and to apply the molecular genetic diagnosis to the clinic.

Abbreviations
DAVID: The database for annotation, visualization and integrated discovery; DEG: Differentially expressed gene; FC: Fold change; FDR: False discovery rate; GO: Gene ontology; NFATC1: Nuclear factor of activated T-cells, cytoplasmic 1; NRP2: Neuroulin-2; OS: Osteosarcoma; PPI: Protein-protein interaction; RANKL: Receptor activator of NF-κB ligand; TCA: Tricarboxylic acid cycle.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
Conceive and design the experiments: ZDC, YQH conceived and designed the experiments. BYD drafted and revised the article, and analyzed and interpreted the data. All authors read and approved the final manuscript.

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References
1. Ottaviani G, Jaffe N: The epidemiology of osteosarcoma. In Pediatric and Adolescent Osteosarcoma. Springer; 2010:13–3.
2. Brodey RS, Riser WH: Canine osteosarcoma: a clinicopathologic study of 194 cases. Clin Orthop Relat Res 1969, 62:54–64.
3. Bacci G, Picci P, Ferrari S, Ruggieri P, Casadei R, Tienghi A, Prever AB, Gherlinzoni F, Mercuri M, Monti C: Primary chemotherapy and delayed surgery for nonmetastatic osteosarcoma of the extremities. Results in 164 patients preoperatively treated with high doses of methotrexate followed by cisplatin and doxorubicin. Cancer 1993, 72:3227–3238.
4. Bacci G, Mercier M, Brilli C, Ferrari S, Bertoni F, Donati D, Monti C, Zanoni A, Forini C, Manfrin M: Osteogenic sarcoma of the extremity with detectable lung metastases at presentation. Cancer 1997, 79:245–254.
5. Marino N, Gebhardt M, Teot L, Gorlick R: Biology and therapeutic advances for pediatric osteosarcoma. Oncologist 2004, 9:422–441.
6. Lu X-Y, Lu Y, Zhao Y-J, Jia Weon K, Kang J, Xiao-Nan L, Ge G, Preyer M, Perlasky L, Hicks J: Cell cycle regulator gene CDCL5, a potential target for 6p12-p21 amplicon in osteosarcoma. Mol Cancer Res 2008, 6:937–946.
7. Song B, Wang Y, Tilmann MA, Botchkina G, Formentini A, Kammann M, Lu J: Research Molecular Mechanism of Chemoresistance by mTOR-215 in Osteosarcoma and Colon Cancer Cells. BMC Cancer 2010.
8. Laverdiere C, Hoang BH, Yang R, Sowers R, Qin JI, Meyers PA, Huivos AG, Healey JH, Gorlick R: Messenger RNA expression levels of CXCR4 correlate with metastatic behavior and outcome in patients with osteosarcoma. Clin Cancer Res 2005, 11:2561–2567.
9. Luu HH, Zhou L, Haydon RC, Deyrup AT, Montag AG, Hui D, Beck R, Heizmann CW, Peabody TD, Simon MA: Increased expression of S100A6 is associated with decreased metastasis and inhibition of cell migration and anchorage independent growth in human osteosarcoma. Cancer Lett 2005, 229:135–148.
10. Kang HG, Kim HS, Kim KJ, Oh JH, Lee MR, Seol SM, Han I: RECK expression in osteosarcoma: correlation with matrix metalloproteinases activation and tumor invasiveness. Journal Orthop Res 2007, 25:696–702.
11. Fisher JL, Mackie PS, Howard ML, Zhou H, Choong PF: The expression of the urorikase plasminogen activator system in metastatic murine osteosarcoma: an in vivo mouse model. Clin Cancer Res 2001, 7:654–660.
12. Vahina C, Khan J, Nguyen P, Perihi J, Caylor J, Yeung C, Trepel J, Melzer P, Helman L: Metastasis-associated differences in gene expression in a murine model of osteosarcoma. Cancer Res 2001, 61:3750–3759.
13. Endo-Munoz L, Cumming A, Rickwood D, Wilson D, Cueva C, Ng C, Stratton G, Cassady A, Evtokiou A, Sommerville S: Loss of osteoclasts contributes to development of osteosarcoma pulmonary metastases. Cancer Res 2010, 70:6063–7072.
14. Troyanskaya O, Cantor M, Sherlock G, Brown P, Hastie T, Tibshirani R, Botstein D, Altman RB: Missing value estimation methods for DNA microarrays. Bioinformatics 2001, 17:520–525.
15. FuJi T, Sato J, Rodrigues L, Ferreira C, Sogayar M: Evaluating different methods of microarray data normalization. BMC Bioinformatics 2006, 7:469.
16. Smyth GK: Limma: linear models for microarray data. In Bioinformatics and Computational Biology Solutions using R and Bioconductor. Springer; 2005:397–420.
17. Dudoit S, Shaffer JP, Boldrick JC: Multiple hypothesis testing in microarray experiments. Stat Sci 2003, 28:1–8.
18. Breitkreutz B-J, Stark C, Tyers M: The GRID: the general repository for interaction datasets. Genome Biol 2003, 4:223.
19. Stark C, Breitkreutz B-J, Reguly T, Boucher L, Breitkreutz A, Tyers M: BioGRID: a general repository for interaction datasets. Nucleic acids research 2006, 34:D535–D539.
20. Willis RC, Hogue CW: Searching, viewing, and visualizing data in the Biomolecular Interaction Network Database (BIND). Curr Protoc Bioinformatics 2006, 8:91–8.
21. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, et al.: Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 2000, 25:25–29.
22. Sherman BT, Lempicki RA: Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic acids research 2009, 37:1–13.
23. Yu G, Wang LG, Han Y, He QY: ClusterProfiler: an R package for comparing biological themes among gene clusters. OMICS 2012, 16:284–287.
24. da Huang W, Sherman BT, Lempicki RA: Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2008, 4:44–57.
25. Zhang B, Kirov S, Snoddy J: WebGestalt: an integrated system for exploring gene sets in various biological contexts. Nucleic Acids Res 2005, 33:W741–W748.
26. Duncan D, Produduturi N, Zhang B: WebGestalt2: an updated and expanded version of the Web-based Gene Set Analysis Toolkit. BMC Bioinformatics 2010, 11:P10.
27. Mullen AR, Wheaton WW, Jin ES, Chen P-H, Cheng T, Yang Y, Healey JH, Formentini A, Kammann M, Lu J: Research Molecular Mechanism of Chemoresistance by mTOR-215 in Osteosarcoma and Colon Cancer Cells. BMC Cancer 2010.
28. Bihani T, Mason DX, Jackson T, Chen SC, Boettner B, Lin AW: Differential oncogenic Ras signaling and senescence in tumor cells. Cell Cycle 2004, 3:1199–1205.
29. Yang DD, Feng S, Chen W, Zhao H, Paulson C, Li YP: V-ATPase subunit ATP6AP1 (Ac5) regulates osteoclast differentiation, extracellular acidification, lysosomal trafficking, and protease exocytosis in osteoclast-mediated bone resorption. J Bone Miner Res 2012, 27:1665–1707.
30. Kim T, Ha HH, Kim N, Yi O, Lee SH, Choy Y: Adm1 interacts with Atpe6vd2 and regulates osteoclast differentiation. Biochem Biophys Res Commun 2009, 390:585–590.
31. Broadhead ML, Clark JC, Dass CR, Choong PF, Myers DE: Therapeutic targeting of osteoclast function and pathways. Expert Opin Ther Targets 2011, 15:169–181.

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32. Kikuta J, Ishii M: Osteoclast migration, differentiation and function: novel therapeutic targets for rheumatic diseases. *Rheumatology* 2013, 52:226–234.

33. Jensen ED, Pham L, Billington CJ, Espe K, Carlson AE, Westendorf JJ, Petryk A, Gopalakrishnan R, Mansky K: Bone morphogenic protein 2 directly enhances differentiation of murine osteoclast precursors. *J Cell Biochem* 2010, 109:672–682.

34. Murthy MB: Osteoimmunology - unleashing the concepts. *J Indian Soc Periodontol* 2011, 15:190.

35. Bielenberg DR, Klagsbrun M: Targeting endothelial and tumor cells with semaphorins. *Cancer Metastasis Rev* 2007, 26:421–431.

36. Kawakami T, Tokunaga T, Hatanaka H, Kijima H, Yamazaki H, Abe Y, Osamura Y, Inoue H, Ueyama Y, Nakamura M: Neuropilin 1 and neuropilin 2 co-expression is significantly correlated with increased vascularity and poor prognosis in nonsmall cell lung carcinoma. *Cancer* 2002, 95:2196–2201.

37. Narazaki M, Tosato G: Tumor cell populations differ in angiogenic activity: a model system for spontaneous angiogenic switch can tell us why. *J Natl Cancer Inst* 2006, 98:294–295.

38. Handa A, Tokunaga T, Tsuchida T, Lee YH, Kijima H, Yarnazaki H, Ueyama Y, Fukuda H, Nakamura M: Neuropilin-2 expression affects the increased vascularization and is a prognostic factor in osteosarcoma. *Int J Oncol* 2000, 17:291–295.

39. Mori K, Berreur M, Blanchard F, Chevalier C, Gisler-Marsollier I, Masson M, Rédimi F, Heymann D: Receptor activator of nuclear factor-kappaB ligand (RANKL) directly modulates the gene expression profile of RANK-positive Saos-2 human osteosarcoma cells. *Oncology Rep* 2007, 18:1365–1371.

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