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Identification of hub genes and pathways associated with retinoblastoma based on co-expression network analysis

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ABSTRACT. The objective of this paper was to identify hub genes and pathways associated with retinoblastoma using centrality analysis of the co-expression network and pathway-enrichment analysis. The co-expression network of retinoblastoma was constructed by weighted gene co-expression network analysis (WGCNA) based on differentially expressed (DE) genes, and clusters were obtained through the molecular complex detection (MCODE) algorithm. Degree centrality analysis of the co-expression network was performed to explore hub genes present in retinoblastoma. Pathway-enrichment analysis was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Validation of hub gene expression in retinoblastoma was performed by reverse transcription-polymerase chain reaction (RT-PCR) analysis. The co-expression network based on 221 DE genes between retinoblastoma and normal controls consisted of 210 nodes and 3965 edges, and 5 clusters of the network were evaluated. By assessing the centrality analysis of the co-

expression network, 21 hub genes were identified, such as SNORD115-41, RASSF2, and SNORD115-44. According to RT-PCR analysis, 16 of the 21 hub genes were differently expressed, including RASSF2 and CDCA7, and 5 were not differently expressed in retinoblastoma compared to normal controls. Pathway analysis showed that genes in 2 clusters were enriched in 3 pathways: purine metabolism, p53 signaling pathway, and melanogenesis. In this study, we successfully identified 16 hub genes and 3 pathways associated with retinoblastoma, which may be potential biomarkers for early detection and therapy for retinoblastoma.

Key words: Retinoblastoma; Co-expression network; Hub gene; Pathway; Reverse transcription-polymerase chain reaction

INTRODUCTION

Retinoblastoma, which originates from the progenitors of retinal sensory cells, is the most common malignant tumor of the eye in children and accounts for about 2-3% of all pediatric malignancies (Villanueva, 2014). Its incidence is approximately 1 in 15,000-20,000 live births each year, and 60% of cases are unilateral (Aerts et al., 2006). The two most common symptoms of retinoblastoma are leukocoria and strabismus, and in addition, iris rubeosis, hypopyon, hyphema, buphthalmia, orbital cellulites, and exophthalmia may also be observed (Benavente and Dyer, 2015). Diagnosis and treatment of retinoblastoma are based on these clinical symptoms and mainly comprise enucleation, external beam radiotherapy, and chemotherapy (Piña et al., 2012).

The success rates in treatment of retinoblastoma vary, with developed countries having the highest success rates; however, most children with retinoblastoma in developing countries die as a result of late diagnosis and poor treatment compliance, which leads to extraocular dissemination and metastasis (Canturk et al., 2010). A recent retrospective series from China and preliminary data from a prospective multicenter study from Central America have shown a survival rate greater than 80% in children with retinoblastoma whose families were at high risk of treatment abandonment if the child was given pre-enucleation chemotherapy (Luna-Fineman et al., 2012). Therefore, early detection and treatment of retinoblastoma is particularly important in developing countries.

Studies of retinoblastoma based on microarray analysis have determined genes involved in the molecular initiation and progression of the disease, which may lead the way in identifying potential molecular biomarkers for early detection of retinoblastoma (Villanueva, 2014). Chakraborty et al. (2011) and (Reis et al., 2012) identified deregulated genes in functional classes and suggested that the phosphoinositide-3-kinase/Akt/mammalian target of Rapamycin/ribosomal S6 kinase 1 signaling pathway was dysregulated in retinoblastoma. However, inconsistent results have been present due to multiple problems, including small sample size, measurement error, and different statistical methods (Ganguly and Shields, 2010). The overlap is very low for the most significantly dysregulated genes across multiple studies (Liang et al., 2012). Network-based approaches, particularly co-expression networks, offer an effective method to at least partially solve this discrepancy by identifying and connecting potential malignancy diagnostic molecular markers. Furthermore, we will gain insight into the important and targetable tumorigenic genes and pathways of retinoblastoma, many of which can be applied to its early detection and treatment (Thériault et al., 2014).

The objective of this paper is to identify hub genes and pathways associated with retinoblastoma using centrality analysis of co-expression networks and pathway-enrichment

analysis. To achieve this, we first constructed a co-expression network using weighted gene co-expression network analysis (WGCNA) based on differentially expressed (DE) genes that were identified by the empirical Bayes approach. Secondly, clusters and degree centrality analysis of the co-expression network were performed. Clusters were obtained using the molecular complex detection (MCODE) algorithm and degree centrality was used to explore hub genes present in retinoblastoma. Pathway-enrichment analysis was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Finally, reverse transcription-polymerase chain reaction (RT-PCR) analysis was conducted to validate the results based on network analysis.

MATERIAL AND METHODS

Dataset selection

Microarray expression profiles of GSE24673 from the gene expression omnibus (GEO) database were selected for identifying DE genes of retinoblastoma. GSE24673, which was already deposited in GPL6244 (Affymetrix Human Gene 1.0 ST Array Platform), consisted of 2 normal healthy adult retina collected from cadaveric eyes and 3 retinoblastoma primary tumors.

Identification of DE genes

DE genes between retinoblastoma patients and normal subjects were identified using the empirical Bayes approach in linear models for microarray data (LIMMA) package. The empirical Bayes approach is equivalent to shrinkage of the estimated sample variances towards a pooled estimate, resulting in far more stable inference when the number of arrays is small (Smyth, 2004). The *t*-test was applied to estimate significance of DE genes between normal subjects and retinoblastoma patients according to the formula (1). The difference between the two groups was assessed by the difference of the means of expression level (equation 2) and normalized by the corresponding SD (equation 3).

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{S_1^2 / n_1 + S_2^2 / n_2}} \quad \text{(Equation 1)}$$

of

$$\bar{X}_i = \sum_{j=1}^{n_i} X_{ij} / n_i \quad \text{(Equation 2)}$$

and

$$S_i^2 = \frac{1}{n_i - 1} \sum_{j=1}^{n_i} (X_{ij} - \bar{X}_i)^2 \quad \text{(Equation 3)}$$

where n_i was the number of repetitions of one condition and x_{ij} was the gene expression level measured in condition *i* repeated *j* times.

The P value, which was obtained using the *t*-test, was tested multiple times by false-discovery rate (FDR) correction based on the Benjamini & Hochberg algorithm. Only the genes which met our criteria ($P < 0.01$, $|\log_2 FoldChange| > 2$) were selected as DE genes in this study.

Construction of the co-expression network

WGCNA has frequently been used to describe correlation patterns among gene expression

profiles (Zhang and Horvath, 2005). Using this method, our first step was to define a measure of similarity between the gene expression profiles. The *n* x *n* similarity matrix $S = [s_{ij}]$ was transformed into the *n* x *n* adjacency matrix $A = [a_{ij}]$, which encoded the connection strength between pairs of nodes. For each pair of genes (x_i and x_j), the similarity measured was represented by S_{ij}. We defined the absolute value of the Pearson correlations ($S_{ij} = |cor(x_i,x_j)|$) of an unsigned network taking on a value between 0 and 1. However, a signed co-expression measure between x_i and x_j was applied to preserve the sign of the correlation which was defined with a simple transformation of the correlation:

$$S_{ij} = \frac{1 + cor(x_i, x_j)}{2} \quad \text{(Equation 4)}$$

The adjacency function was used to determine the adjacency matrix $A = [a_{ij}]$. The most widely used adjacency function was the signum function, which implements "hard" thresholds involving the threshold parameter τ:

$$a_{ij} = signum(s_{ij}, \tau) = \begin{cases} 1 & if\ S_{ij} \geq \tau \\ 0 & otherwise \end{cases} \quad \text{(Equation 5)}$$

The hard threshold may lead to a loss of information; therefore a "soft" adjacency function was needed. For this, we used the power adjacency function: $a_{ij} = |s_{ij}|^{\beta}$ with the single parameter β.

As for the overlap of two nodes, which reflects their relative interconnectedness, the topological overlap matrix (TOM; (TOM) $\Omega = [\omega_{ij}]$) provided a similarity measure. For a dissimilarity measure, the difference between 1 and this value was used (i.e, the topological overlap-based dissimilarity measure was defined as $d_{ij}^{\omega} = 1 - \omega_{ij}$). In addition, the weight value threshold of the co-expression network was 0.7.

Analysis of the co-expression network

Degree centrality analysis

To evaluate DE genes in a biological network, the degree centrality of the network was computed. The degree of a node (gene or protein) is the average number of edges (interactions) incident to this node. The degree quantifies the local topology of each gene by summing up the number of its adjacent genes (Haythornthwaite, 1996). The degree *C(v)* of a node *v* was defined as:

$$C(v) = \sum_{j} a_{vj} \quad \text{(Equation 6)}$$

This gives a simple count of the number of interactions of a given node. The genes at the top of the degree distribution (≥90% percentile) in the significantly perturbed networks were defined as hub genes.

Sub-network analysis

In this paper, the MCODE algorithm was used to analyze a subset of the co-expression network (Bader and Hogue, 2003). Vertex weighting, complex prediction, and optional post-processing were the three main stages of the MCODE algorithm. At the vertex weighting stage, all vertices based on their local network density were weighted using the highest *k*-core of the vertex neighborhood. Next, using the vertex weighted graph as input, a complex with the highest weighted vertex was seeded and moved outward from the seed vertex recursively. It included vertices in the complex whose weight was above a given threshold, which was a given percentage away from the weight of the seed vertex. In this way, the densest regions of the network were identified. In the third stage, complexes with a core less than 2 (graph of minimum degree 2) were filtered, and fluff and haircut options were run. The fluff option was used to increase the size of the complex according to a given fluff parameter between 0 and 1. Using the haircut option, the resulting complexes were 2-cored, thereby removing the vertices which were singly connected to the core complex. When both options were performed, fluff was run first, followed by haircut. Clusters with the thresholds node density cutoff = 1, node score cutoff = 0.2, *k*-core = 2, and maximum depth = 100 were chosen for further analysis.

Pathway-enrichment analysis

Pathway-enrichment analysis of nodes from existing clusters of hub genes was conducted based on the KEGG database (Kanehisa and Goto, 2000). KEGG pathways with a P value less than 0.1 were selected according to the expression analysis systematic explored (EASE) test implemented in the database for annotation, visualization and integrated discovery (DAVID) (Grossglauser and Vetterli, 2003). The principle of EASE was used as follows:

$$P = \frac{\binom{a+b}{a}\binom{c+d}{c}}{\binom{n}{a+c}} \quad \text{(Equation 7)}$$

where $n = a' + b + c + d$ was the number of background genes; a' was the number of genes in one gene set in the gene list; $a' + b$ was the number of genes in the gene list including at least one gene set; $a' + c$ was the number of genes in one gene list in the background genes; a' was replaced with $a = a' - 1$.

Validation of hub genes by RT-PCR analysis

Samples from 10 patients with retinoblastoma were collected, and RNA was prepared from retinoblastoma cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The RNA was used in first-strand synthesis with the dT18 primer and was treated with 40 U/μL RNasin, 1X reverse transcriptase buffer, 4 mM dNTPs, and 5 U/μL AMV reverse transcriptase according to the manufacturer instructions (Invitrogen). For RT-PCR, each reaction contained 1X PCR Buffer I, 2.5 U/μg High Fidelity Taq DNA Polymerase, 0.5 μM of each forward and reverse primer, and 4 mM dNTPs. The cDNA from each sample was used as template, with β-actin as an internal reference. Primer sequences to amplify the 21 hub genes are listed in Table 1. Conditions were as follows:

2 min at 94°C for predenaturation, followed by 35 cycles of 20 s at 94°C, 15 s at 60°C and 1 min at 68°C, and a final 7 min extension at 72°C. Products of the PCR reaction were analyzed by electrophoresis on 1.5% agarose gels and imaged using Quantity One Software (Bio-Rad, USA).

Table 1. Sequences of primers and RT-PCR product lengths for 21 candidate genes.

Gene	Primers (5'-3')		Length (bp)
	Forward	Reverse	
SNORD115-41	TGATGAGAACCTTCTATTGTCCTGA	GGGCCTCAGCGTAATCCTAT	75
RASSF2	AAAAAGTTGGCACCATGGAC	AGCCATAGGCTGGTGTGAAC	579
SNORD115-44	AGTGATGAGAACCTTCTATTGTCCT	GGGCCTCAGCGTAATCCTATT	77
CCDC60	GATTCGACCCCATGTCCTCC	TACAAGAAAGTTGGGTACCTCAGGG	201
CDCA7	TGGAGAACGTCTGCAGCAAT	TACAAGAAAGTTGGGTATGCTTGC	382
UBE2T	TGCAGAGAGCTTCACGTCTG	GAGGGATGGTCTCCAAGCAC	305
NEK2	TGGCTCCATGACAGAAGCTG	TTGTACAAGAAAGTTGGGTAAAAGA	540
PROX1	GAGGGTGGGAAAGGGGTTTT	TCAAACGGCACTGAGCTTGT	204
HIST1H3B	GACCGAGTTGCTGATTCGGA	ACAAGAAAGTTGGCAACGC	251
NT5E	TTTTGCACACCAACGACGTG	TGGCCCATGCAATCCTTTTG	690
ELMOD1	AGCAGGTCCTGTCTGACTCT	TACAAGAAAGTTGGGTACATGTTG	433
PAX6	CAGTATAAGCGGGAGTGCCC	ATCGTTGGTACAGACCCCCT	81
ANK3	TGGGCCACTGCTTTGTTTTC	GGCAGGGAATCATCACCCAA	693
TMEM123	AAGGGTTTGGTTCGGGGTTT	TTGTGTGAGCAACAAAGGCG	225
CCDC141	CCAGGCTGCTGTCCAATGTA	TGTGTATGTTGGGGGAGTGC	457
GEM	GAGAAGGGAGAGAGTGGGAGT	CTCGCTCGGTCTGTGATTGA	593
HIST1H1B	ACGGCATTGCAAAACTTGCT	AAAGGCCATTGCGCTCTTTG	247
TIAM2	TTTGCAGATCTGGGCGTGAT	CCTCGTCCGTGTTCTTGTCA	256
SPP1	AGCAGAATCTCCTAGCCCCA	ACGGCTGTCCCAATCAGAAG	467
SNORD115-14	TGAGAACCTTCTATTGTCCTGAAGA	GGCCTCAGCGTAATCCTATTGA	71
NAAA	GTGGCTCAAGACTCCAGAGG	AAGAAAGTTGGGCACTCAGGG	224

RESULTS

Identification of DE genes

We obtained 221 DE genes, including 83 upregulated genes and 138 downregulated genes, between retinoblastoma patients and normal subjects. Specifically, SLC4A10 ($P = 3.60e^{-12}$), FAIM2 ($P = 5.65e^{-11}$), PAX6 ($P = 6.57e^{-11}$), PVALB ($P = 6.87e^{-11}$) and GABRG1 ($P = 6.98e^{-11}$) were the most significant DE genes.

Co-expression network analysis

The co-expression network of the 221 DE genes in retinoblastoma 221 DE genes constructed by WGCNA is shown in Figure 1. There were 210 nodes and 3965 edges in the co-expression network with a threshold weight of > 0.7. Genes in the 90th or greater percentile distribution in the network were defined as hub genes. By assessing degree centrality of the co-expression network, we obtained 21 hub genes (Table 2), where SNORD115-41(degree = 135), RASSF2 (degree = 129), SNORD115-44 (degree = 127), CCDC60 (degree = 125) and CDCA7 (degree = 125) were the top five hub genes.

We used the MCODE algorithm to analyze a subset of the co-expression network. When setting the node density cutoff to 1, the node score cutoff to 0.2, the *k*-core to 2, and the maximum depth to 100, five clusters were identified and are shown in Figure 2. We found that there were 9 hub genes in Cluster 1 (SNORD115-14, SPP1, CCDC141, GEM, ANK3, RASSF2, TMEM123, SNORD115-41 and SNORD115-44) and 12 hub genes in Cluster 2 (NT5E, NEK2,

CCDC60, PROX1, CDCA7, HIST1H3B, NAAA, UBE2T, PAX6, ELMOD1, TIAM2 and HIST1H1B). The number of hub genes in the two clusters was equal to the total hub genes obtained from the co-expression network. There were no hub genes present in Clusters 3, 4 or 5.

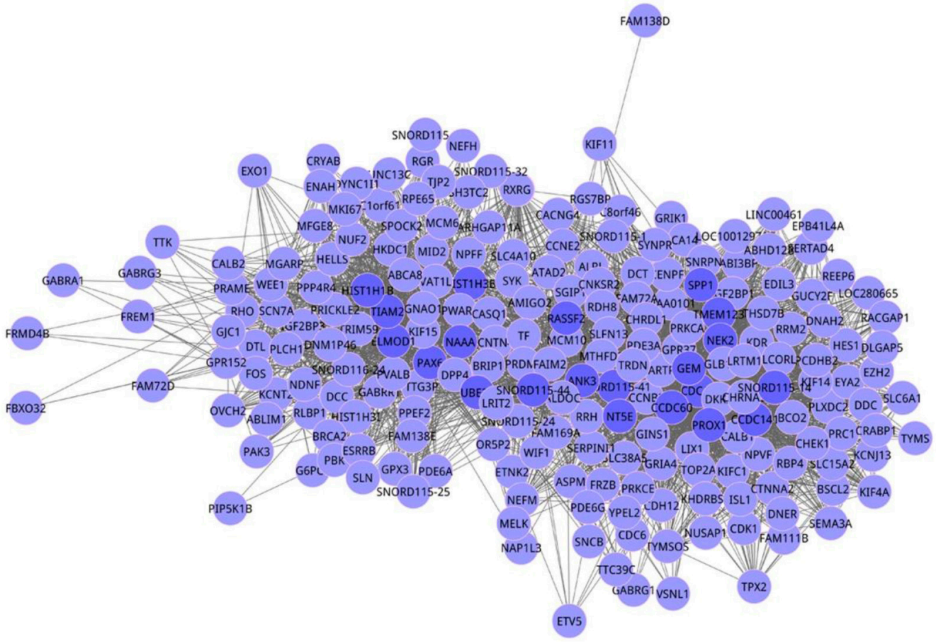

Figure 1. Co-expression network of retinoblastoma based on 221 DE genes. There were 210 nodes and 3965 edges in the co-expression network, where nodes (circles) are genes and edges between nodes (gray lines) indicate the interaction of genes in the network. The darker blue genes represent hub genes.

Table 2. Hub genes in the co-expression network.

No.	Gene	Degree
1	*SNORD115-41*	135
2	*RASSF2*	129
3	*SNORD115-44*	127
4	*CCDC60*	125
5	*CDCA7*	125
6	*UBE2T*	114
7	*NEK2*	108
8	*PROX1*	103
9	*HIST1H3B*	93
10	*NT5E*	93
11	*ELMOD1*	92
12	*PAX6*	90
13	*ANK3*	88
14	*TMEM123*	88
15	*CCDC141*	86
16	*GEM*	85
17	*HIST1H1B*	83
18	*TIAM2*	83
19	*SPP1*	83
20	*SNORD115-14*	82
21	*NAAA*	81

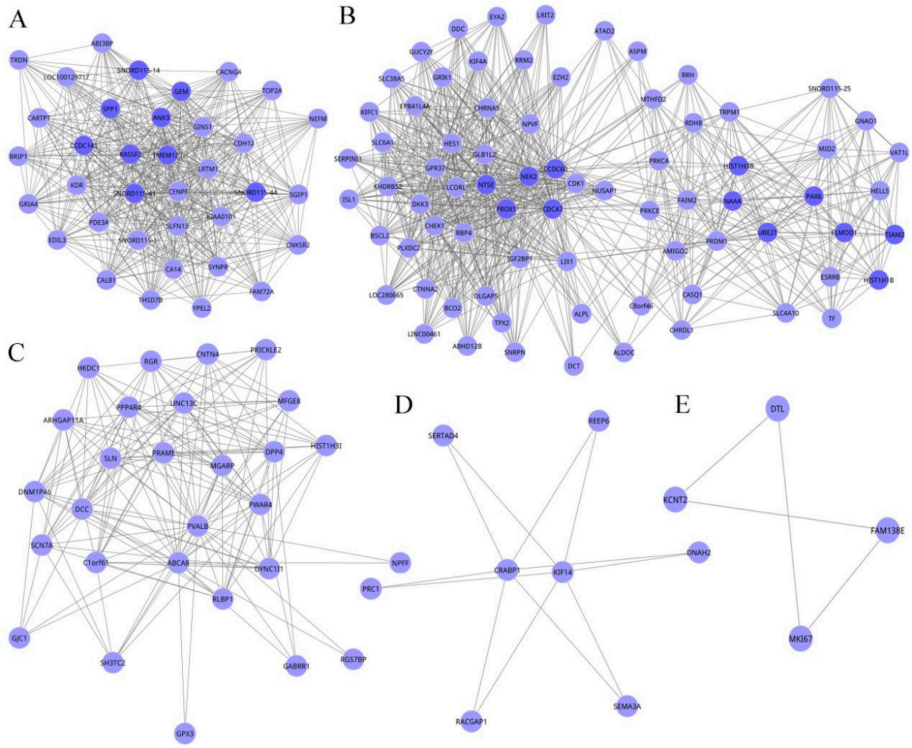

Figure 2. Clusters of genes in co-expression network in retinoblastoma. **A** is Cluster 1, **B** is Cluster 2, **C** is Cluster 3, **D** is Cluster 4, and **E** is Cluster 5. Clusters were achieved using the conditions of node density cutoff = 1, node score cutoff = 0.2, k-core = 2, and maximum depth = 100.

Pathway-enrichment analysis

The KEGG database is a collection of manually drawn pathway maps for metabolism, genetic information processing, and environmental information processing, including signal transduction and various other cellular processes, and how these pathways are affected in human diseases. In this paper, KEGG pathway-enrichment analysis of genes in Clusters 1 and 2, which consisted of hub genes, showed that purine metabolism ($P = 0.046$), p53 signaling pathway ($P = 0.0499$) and melanogenesis ($P = 0.0962$) were significantly affected pathways when the condition of $P < 0.1$ was applied.

Validation of hub genes

To confirm the results of network-based analysis and investigate key genes in retinoblastoma, the relative expression levels of 21 hub genes from the co-expression network were analyzed by RT-PCR (Figure 3). We found that 16 of the 21 hub genes were differently expressed in retinoblastoma compared to normal controls (RASSF2, CDCA7, UBE2T, PROX1, NT5E, ELMOD1, PAX6, CCDC141, GEM, TIAM2 and SPP1 with $P < 0.001$; SNORD115-41, SNORD115-44, HIST1H3B, HIST1H1B and SNORD115-14 with $P > 0.05$).

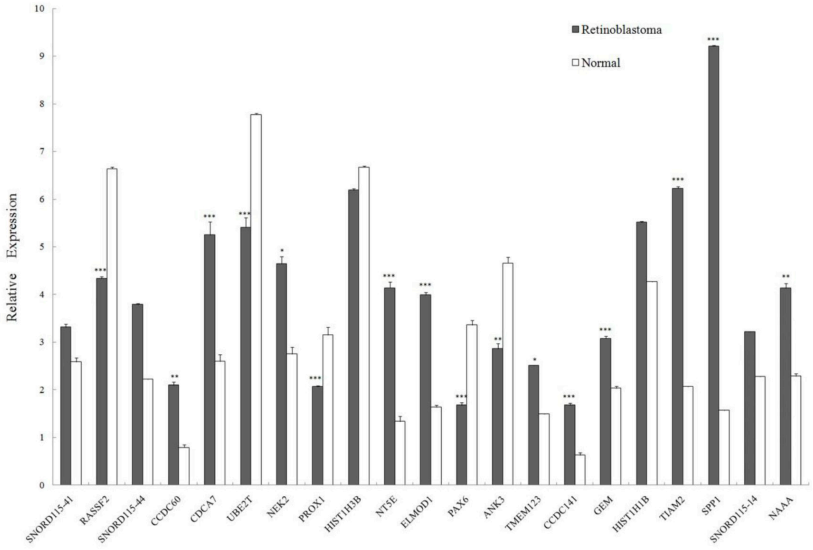

Figure 3. Relative expression levels of 21 hub genes. The expressions of genes in retinoblastoma (gray bars) compared to normal controls (white bars) are shown and significance is indicated by P value: *0.01 < P < 0.05; **0.001 < P < 0.01; and ***P < 0.001.

DISCUSSION

In this study, we identified important genes and pathways in retinoblastoma based on degree centrality analysis of a co-expression network and pathway-enrichment analysis. We identified 21 hub genes, such as SNORD115-41, RASSF2 and SNORD115-44, by conducting centrality analysis of the co-expression network and its 5 clusters according to the MCODE algorithm, respectively. In addition, RT-PCR was carried out to verify network-based results and to investigate genes differently expressed in retinoblastoma. Purine metabolism, p53 signaling pathway and melanogenesis were pathways significantly affected in retinoblastoma.

RT-PCR analysis showed that 16 of the 21 hub genes identified in the co-expression network were differently expressed, including RASSF2 and CDCA7, while 5 were not differently expressed, including SNORD115-41 and SNORD115-44, in retinoblastoma compared to normal controls. This result was not entirely consistent with our network analysis but most of the hub genes were differently expressed in retinoblastoma patients. The probable reason for this was variations in samples, as the microarray data was downloaded from the GEO database while the RT-PCR was conducted by our group. Therefore, using network analysis and RT-PCR, we have identified 16 potential biomarkers of retinoblastoma.

RASSF2, Ras association domain-containing protein 2, is a pro-apoptotic Ras effecter that is frequently downregulated in human tumors by promoter methylation, histone deacetylation, and occasionally deletion (Donninger et al., 2007). Hesson et al. (2005) revealed that RASSF2 inactivation correlated with activation of Ras in tumor cells. Furthermore, knocking down RASSF2 enhanced tumorigenesis, further proving that RASSF2 is an epigenetically inactivated tumor suppressor (Akino et al., 2005). It was shown that RASSF2 was widely expressed and its overexpression inhibited growth of tumor cell lines (Agathanggelou et al., 2005). More specifically,

RASSF2 inhibited cell growth and induced cell death when co-expressed with activated K-Ras (Agathanggelou et al., 2005). We conclude that RASSF2 is a tumor suppressor and might also be a potential biomarker for retinoblastoma.

Cell division cycle associated 7 (CDCA7) was a recently identified target of myc-dependent transcriptional regulation and is a proto-oncogene that regulates the expression of hundreds of genes involved in cell cycle progression, adhesion, metabolism, and apoptosis (Guiu et al., 2014). CDCA7 induced colony formation and cell proliferation, indicating that it played an important role in tumor development (Tian et al., 2013). High levels of CDCA7 mRNA have been found in patients with acute myeloid leukemia and it was strongly upregulated in human hepatocellular carcinoma (Gill et al., 2013). There have been a few of studies focused on CDCA7 expression in retinoblastoma. For example, Goto et al. (2006) related CDCA7 expression with downstream components of a growth regulatory pathway that was also affected in retinoblastoma. In our work, we confirmed that CDCA7 is involved in retinoblastoma.

In this paper, we explored three significant pathways of retinoblastoma: purine metabolism, p53 signaling pathway and melanogenesis. P53 is a sequence-specific DNA-binding protein that promotes cell-cycle arrest or apoptosis in response to a variety of cellular stresses. The high frequency of p53 mutations in human cancer implies a central role for p53 in tumorigenesis, and the p53 signaling pathway has been described as a cellular surveillance mechanism for cancer prevention (Chakraborty et al., 2011). Molecular elucidation of p53 signaling unraveled novel signaling concepts, broadening our understanding of p53 function and illustrating the importance of p53 signaling in the pathogenesis and treatment of cancer (Stegh, 2012). In addition, Mirzayans et al. (2012) demonstrated that ionizing radiation triggered sequential waves in the p53 signaling pathway of human cancer. Retinoblastoma is a human malignant tumor and strongly linked to the p53 signaling pathway.

In conclusion, we successfully identified 21 hub genes and 3 pathways in retinoblastoma based on co-expression network analysis, and confirmed that 16 of the 21 hub genes were differently expressed in retinoblastoma using RT-PCR assays. These genes and pathways could be potential biomarkers for early detection and therapy for retinoblastoma.

Conflicts of interest

The authors declare no conflict of interest.

REFERENCES

Aerts I, Lumbroso-Le Rouic L, Gauthier-Villars M, Brisse H, et al. (2006). Retinoblastoma. *Orphanet. J. Rare Dis.* 1: 31.

Agathanggelou A, Cooper WN and Latif F (2005). Role of the Ras-association domain family 1 tumor suppressor gene in human cancers. *Cancer Res.* 65: 3497-3508.

Akino K, Toyota M, Suzuki H, Mita H, et al. (2005). The Ras effector RASSF2 is a novel tumor-suppressor gene in human colorectal cancer. *Gastroenterology* 129: 156-169.

Bader GD and Hogue CW (2003). An automated method for finding molecular complexes in large protein interaction networks. *BMC Bioinformatics* 4: 2.

Benavente CA and Dyer MA (2015). Genetics and epigenetics of human retinoblastoma. *Annu. Rev. Pathol.* 10: 547-562.

Canturk S, Qaddoumi I, Khetan V, Ma Z, et al. (2010). Survival of retinoblastoma in less-developed countries impact of socioeconomic and health-related indicators. *Br. J. Ophthalmol.* 94: 1432-1436.

Chakraborty A, Uechi T and Kenmochi N (2011). Guarding the 'translation apparatus': defective ribosome biogenesis and the p53 signaling pathway. *Wiley Interdiscip. Rev. RNA* 2: 507-522.

Donninger H, Vos MD and Clark GJ (2007). The RASSF1A tumor suppressor. *J. Cell Sci.* 120: 3163-3172.

Ganguly A and Shields CL (2010). Differential gene expression profile of retinoblastoma compared to normal retina. *Mol. Vis.* 16: 1292.

Gill RM, Gabor TV, Couzens AL and Scheid MP (2013). The MYC-associated protein CDCA7 is phosphorylated by AKT to regulate MYC-dependent apoptosis and transformation. *Mol. Cell. Biol.* 33: 498-513.

Goto Y, Hayashi R, Muramatsu T, Ogawa H, et al. (2006). JPO1/CDCA7, a novel transcription factor E2F1-induced protein, possesses intrinsic transcriptional regulator activity. *Biochim. Biophys. Acta* 1759: 60-68.

Grossglauser M and Vetterli M (2003). Locating nodes with EASE: Last encounter routing in ad hoc networks through mobility diffusion. Presented at INFOCOM 2003, 22nd Annual Joint Conference of the IEEE Computer and Communications, IEEE Societies. San Franciso, CA, USA

Guiu J, Bergen DJ, De Pater E, Islam AB, et al. (2014). Identification of Cdca7 as a novel Notch transcriptional target involved in hematopoietic stem cell emergence. *J. Exp. Med.* 211: 2411-2423.

Haythornthwaite C (1996). Social network analysis: An approach and technique for the study of information exchange. *Libr. Inform. Sci. Res.* 18: 323-342.

Hesson LB, Wilson R, Morton D, Adams C, et al. (2005). CpG island promoter hypermethylation of a novel Ras-effector gene RASSF2A is an early event in colon carcinogenesis and correlates inversely with K-ras mutations. *Oncogene* 24: 3987-3994.

Kanehisa M and Goto S (2000). KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* 28: 27-30.

Liang D, Han G, Feng X, Sun J, et al. (2012). Concerted perturbation observed in a hub network in Alzheimer's disease. *PLoS One* 7: e40498.

Luna-Fineman S, Alejos A, Amador G, Barnoya M, et al. (2012). Pre-enucleation chemotherapy in advanced intraocular retinoblastoma. Central America Experience: AHOPCA II. Presented at the Pediatric Blood and Cancer Meeting, London, United Kingdom.

Mirzayans R, Andrais B, Scott A and Murray D (2012). New insights into p53 signaling and cancer cell response to DNA damage: implications for cancer therapy. *J. Biomed Biotechnol.* 2012: 170325.

Piña Y, Decatur C, Murray TG, Houston SK, et al. (2012). Retinoblastoma treatment: utilization of the glycolytic inhibitor, 2-deoxy-2-fluoro-d-glucose (2-FG), to target the chemoresistant hypoxic regions in LH(BETA)T(AG) retinal tumors. *Invest. Ophthalmol. Vis. Sci.* 53: 996-1002.

Reis AH, Vargas FR and Lemos B (2012). More epigenetic hits than meets the eye: microRNAs and genes associated with the tumorigenesis of retinoblastoma. *Front. Genet.* 3: 284.

Smyth GK (2004). Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat. Appl. Genet. Mol. Biol.* 3: Article 3.

Stegh AH (2012). Targeting the p53 signaling pathway in cancer therapy-the promises, challenges and perils. *Expert Opin. Ther. Targets* 16: 67-83.

Thériault BL, Dimaras H, Gallie BL and Corson TW (2014). The genomic landscape of retinoblastoma: a review. *Clin. Experiment. Ophthalmol.* 42: 33-52.

Tian Y, Huang C, Zhang H, Ni Q, et al. (2013). CDCA7L promotes hepatocellular carcinoma progression by regulating the cell cycle. *Int. J. Oncol.* 43: 2082-2090.

Villanueva MT (2014). Tumorigenesis: Establishing the origin of retinoblastoma. *Nat. Rev. Cancer* 14: 706-707.

Zhang B and Horvath S (2005). A general framework for weighted gene co-expression network analysis. *Stat. Appl. Genet. Mol. Biol.* 4: Article 17.