RESEARCH ARTICLE

Loss of Heterozygosity at the Calcium Regulation Gene Locus on Chromosome 10q in Human Pancreatic Cancer

Jin Long*, Zhong-Bo Zhang, Zhe Liu, Yuan-Hong Xu, Chun-Lin Ge

Abstract

Background: Loss of heterozygosity (LOH) on chromosomal regions is crucial in tumor progression and this study aimed to identify genome-wide LOH in pancreatic cancer. Materials and Methods: Single-nucleotide polymorphism (SNP) profiling data GSE32682 of human pancreatic samples snap-frozen during surgery were downloaded from Gene Expression Omnibus database. Genotype console software was used to perform data processing. Candidate genes with LOH were screened based on the genotype calls, SNP loci of LOH and dbSNP database. Gene annotation was performed to identify the functions of candidate genes using NCBI (the National Center for Biotechnology Information) database, followed by Gene Ontology, INTERPRO, PFAM and SMART annotation and UCSC Genome Browser track to the unannotated genes using DAVID (the Database for Annotation, Visualization and Integration Discovery). Results: The candidate genes with LOH identified in this study were *MCU*, *MICU1* and *OIT3* on chromosome 10. *MCU* was found to encode a calcium transporter and *MICU1* could encode an essential regulator of mitochondrial Ca^{2+} uptake. *OIT3* possibly correlated with calcium binding revealed by the annotation analyses and was regulated by a large number of transcription factors including *STAT*, *SOX9*, *CREB*, *NF-kB*, *PPARG* and *p53*. Conclusions: Global genomic analysis of SNPs identified *MICU1*, *MCU* and *OIT3* with LOH on chromosome 10, implying involvement of these genes in progression of pancreatic cancer.

Keywords: pancreatic cancer - loss of heterozygosity - single-nucleotide polymorphism

Asian Pac J Cancer Prev, **16 (6)**, 2489-2493

Introduction

Loss of heterozygosity (LOH) is a common genetic alteration in cancer genomes, which derives from heterozygous deletion of one of the two alleles, or duplication of a maternal or paternal chromosome or chromosomal region and concurrent loss of the other allele (Frampton and King, 2013). LOH on chromosomal regions containing key tumor suppressor genes has been considered as a significant contributor to drive cancer progression by inactivating the suppressor genes of tumor (Baker et al., 2009), including pancreatic cancer. For example, *p53* tumor suppressor gene is identified to experience LOH accompanied by mutation in pancreatic cancer cell lines (Butz et al., 2003). Cooperating with mutation and/or loss of *p53*, the LOH of *BRCA1* and *BRCA2* may contribute to the progression of pancreatic cancer (Lucas et al., 2013). Besides, LOH in *THBS2* (6q), *p16* (9p) and *APC* (5q) are also demonstrated to carry the worst prognosis for resected pancreatic ductal and ampullary adenocarcinomas (Franko et al., 2008). By using genome-wide LOH maps in pancreatic cancer, LOH is found with high frequency at various chromosomes such as 3p, 6p, 6q, 8q, 9p, 9q, 12q, 13q, 17p, 18q and 22q (Lin et al., 2008). However, candidate genes with LOH in pancreatic cancer remains rarely investigated.

Hybridization to single-nucleotide polymorphism (SNP) arrays is an efficient method to detect genome-wide cancer LOH by identifying the absence of heterozygous loci (Beroukhim et al., 2006; Staaf et al., 2008). Thus, the goal of this study was to further identify candidate genes with LOH in pancreatic cancer by performing a genome-wide analysis of LOH using the SNP arrays deposited in public database by Donahue et al. (2012), followed by annotation analysis and transcription factors screening in an attempt to explain the involvement of these genes in pancreatic cancer.

Materials and Methods

SNP expression profiling data

The SNP expression profiling data GSE32682 (Donahue et al., 2012) of human pancreatic samples including 25 human pancreatic cancer and 7 non-malignant pancreas samples snap-frozen during surgery were downloaded from the GEO (Gene Expression

*Department of General Surgery, the First Hospital of China Medical University, Shenyang, China *For correspondence: jinlongjl18@163.com*

Omnibus) database that is developed as a repository of microarrays, chips, hybridization arrays and high throughput gene expression data. The gene expressions of these samples were investigated by Affymetrix Genome-Wide Human SNP 6.0 Array (GPL6801).

Data processing and screening of candidate genes with LOH

Affymetrix CEL files were analyzed using Genotype Console software (version 4.0; Affymetrix) for initial intensity quality control (QC) with the criterion of recommended contrast QC>0.4, followed by generating SNP genotype calls using the Affymetrix Birdseed algorithm and filtrating SNPs loci with the excluded thresholds of no-call rate ≥ 10%, minor allele frequency <0.05 and Hardy Weinberg Equilibrium (HWE) P-value 0.001. Then, copy-neutral LOH (CN-LOH) analysis was performed with the threshold of MAPD<0.04 to identify the SNP loci of LOH that existed in over 50% of the pancreatic cancer samples while not in control samples. The candidate genes with LOH were screened based on the genotype calls, SNP loci of LOH and dbSNP database (Day, 2010).

Gene annotation of Candidate genes

The candidate genes with LOH were annotated by using Gene database in NCBI (the National Center for Biotechnology Information) and the unannotated genes were inputted into DAVID (the Database for Annotation, Visualization and Integration Discovery) for Gene Ontology (GO), INTERPRO, PFAM and SMART annotation (Sherman et al., 2007).

Transcription factors screening of unannotated genes

UCSC Genome Browser track that displays all analyzed transcription factor binding sites (TFBS) was performed to identify the transcription factors of the unannotated genes using DAVID database (Fujita et al., 2010), which was then visualized by constructing regulatory network using Cytoscape software (Kohl et al., 2011).

Results

Intensity QC filtration

The intensity QC of each sample was analyzed using Genotype Console software (Figure 1). The samples with contrast QC<0.4 (GSM811149_17T.CEL and GSM811154_14T.CEL) were excluded in the following research.

Candidate genes with LOH

The candidate genes with LOH identified in this study were *MCU* (mitochondrial Ca^{2+} uniporter), *MICU1* (mitochondrial calcium uniporter regulator 1) and *OIT3* (oncoprotein induced transcript 3) on chromosome 10

Table 1. Partial results of LOH_SNP_gene

Probe_id	Chr	Pos	CN_state	SNP_ID	Gene_ID
SNP_A-8465056	10	74321221	2	rs7921361	*MICU1*
SNP_A-2002554	10	74334758	2	rs6415911	*MICU1*
SNP_A-8423526	10	74378437	2	rs10823937	*MICU1*
SNP_A-8716423	10	74417164	2	rs9415071	-
SNP_A-1882206	10	74442817	2	rs3009560	-
SNP_A-8631561	10	74490023	2	rs2921448	*MCU*
SNP_A-8397978	10	74512727	2	rs2894214	*MCU*
SNP_A-2163625	10	74514615	2	rs7086453	*MCU*
SNP_A-2120808	10	74522284	2	rs16930097	*MCU*
SNP_A-2027945	10	74522494	2	rs16930102	*MCU*
SNP_A-2140867	10	74541864	2	rs11000413	*MCU*
SNP_A-2297049	10	74570913	2	rs7097134	*MCU*
SNP_A-8486874	10	74606261	2	rs12785228	*MCU*
SNP_A-4259678	10	74672399	2	rs11000445	*OIT3*
SNP_A-8465056	10	74321221	2	rs7921361	*MICU1*
SNP_A-2002554	10	74334758	2	rs6415911	*MICU1*
SNP_A-8465056	10	74321221	4	rs7921361	*MICU1*
SNP_A-2002554	10	74334758	4	rs6415911	*MICU1*
SNP_A-8423526	10	74378437	4	rs10823937	*MICU1*
SNP_A-8716423	10	74417164	4	rs9415071	-
SNP_A-1882206	10	74442817	4	rs3009560	-
SNP_A-8631561	10	74490023	4	rs2921448	*MCU*
SNP_A-8397978	10	74512727	4	rs2894214	*MCU*
SNP_A-2163625	10	74514615	4	rs7086453	*MCU*
SNP_A-2120808	10	74522284	4	rs16930097	*MCU*
SNP_A-2027945	10	74522494	4	rs16930102	*MCU*
SNP_A-2140867	10	74541864	4	rs11000413	*MCU*
SNP_A-2297049	10	74570913	4	rs7097134	*MCU*
SNP_A-8486874	10	74606261	4	rs12785228	*MCU*
SNP_A-4259678	10	74672399	4	rs11000445	*OIT3*

Chr, chromosome; Pos, the loci of SNP on chromosome; CN_state, the copy numbers; SNP_ID, the ID of SNP in dbSNP database; Gene_ID, the ID of gene corresponding to the SNP; LOH, loss of heterozygosity; SNP, single-nucleotide polymorphism

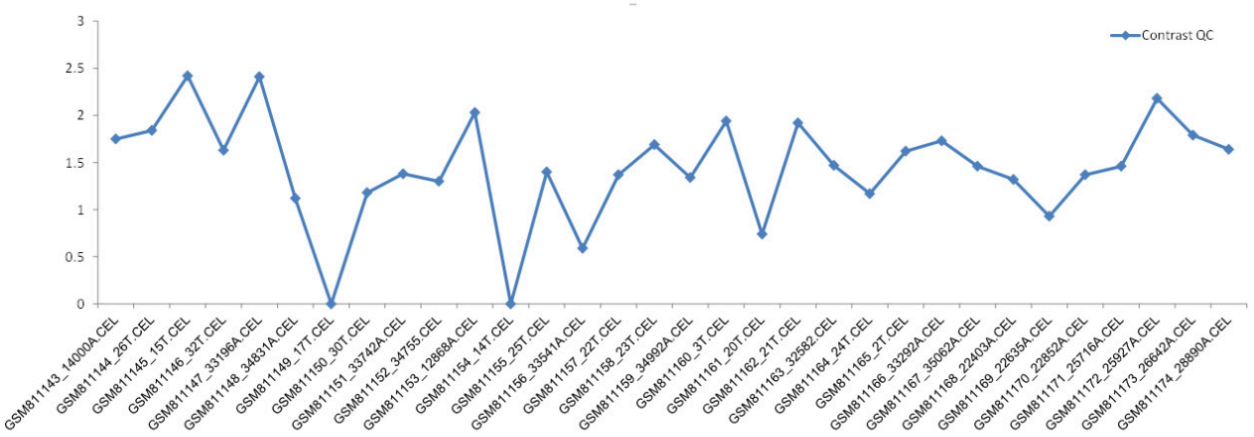

Figure 1. The Intensity QC in Each Sample. The X-axis represents file names of samples and the Y-axis represents the value of contrast QC. QC, quality control

Table 2. The Annotation Results of *OIT3*

ID	*OIT3*
Gene Name	oncoprotein induced transcript 3
GOTERM_CC_FAT	GO:0005635~nuclear envelope,GO:0012505~endomembrane system,GO:0031967~organelle envelope,GO:0031975~envelope
GOTERM_MF_FAT	GO:0005509~calcium ion binding,GO:0043167~ion binding,GO:0043169~cation binding,GO:0046872~metal ion binding
INTERPRO	IPR000152:EGF-type aspartate/asparagine hydroxylation conserved site,IPR001507:Endoglin/CD105 antigen,IPR001881:EGF-like calcium-binding,IPR006209:EGF,IPR006210:EGF-like,IPR013032:EGF-like region, conserved site,IPR017976:Endoglin/CD105 antigen subgroup,IPR017977:Endoglin/CD105 antigen conserved site, IPR018097:EGF-like calcium-binding, conserved site
PFAM	PF00008:227-262,PF00008:EGF,PF00100:Zona pellucida-like domain,PF00100:Zona_pellucida
SMART	SM00179:EGF_CA,SM00181:EGF,SM00241:ZP

*GO, Gene Ontology; CC, cellular component; MF, molecular function

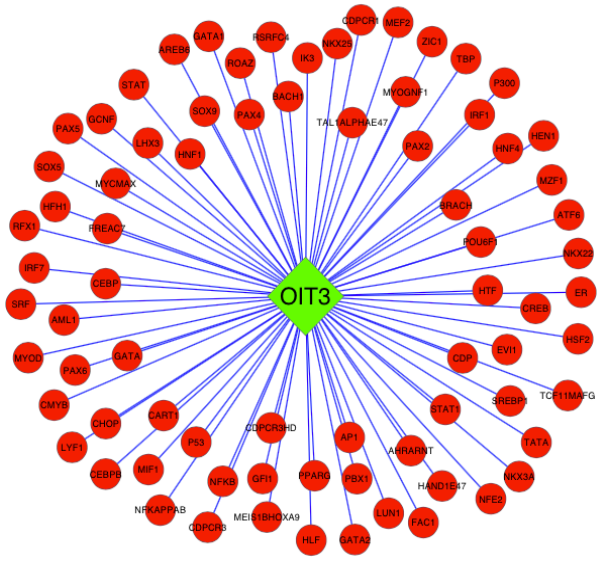

Figure 2. The Regulatory Network Between *OIT3* and its Transcription Factors. The rhombus represents *OIT3* and the surrounding circles represent transcription factors

(Partial results were shown in Table 1). The copy number (CN) in the samples were two or four in this study, probably suggesting LOH resulted from heterozygous deletion of one of the two alleles, or duplication of a maternal or paternal chromosome or chromosomal region and concurrent loss of the other allele.

Gene annotation

Gene annotation was performed to identify the functions of candidate genes with LOH using Gene database in NCBI. Accordingly, *MCU* (Gene ID: 90550) was found to encode a calcium transporter that localizes to the mitochondrial inner membrane and interacts with mitochondrial calcium uptake (Bick et al., 2012; Csordas et al., 2012; Raffaello et al., 2012; Curry et al., 2013; Patron et al., 2013). *MICU1* (Gene ID: 10367) could encode an essential regulator of mitochondrial Ca^{2+} uptake under basal conditions. The encoded protein interacts with the mitochondrial calcium uniporter and is essential in preventing mitochondrial Ca^{2+} overload that could cause excessive production of reactive oxygen species and cell stress (Mallilankaraman et al., 2012; Arvizo et al., 2013; Chen et al., 2013; Hoffman et al., 2013; Logan et al., 2014). *OIT3* didn't obtain any annotated results from Gene database, but get some other annotated messages from GO, INTERPRO, PFAM, SMART annotation analysis provided by DAVID (Table 2). *OIT3*, oncoprotein induced transcript 3, was correlated with calcium ion binding, ion binding and cation binding.

Transcription factors of OIT3

By performing UCSC TFBS analysis, this study identified a large number of transcription factors including *STAT* (signal transducer and activator of transcription), *SOX9* (sex determining region Y-box 9), *CREB* (cAMP responsive element binding protein), *NF-kB* (nuclear factor kappa B), *PPARG* (peroxisome proliferator-activated receptor gamma) and *p53*, which had regulatory effects on *OIT3* (Figure 2).

Discussion

By mapping the SNPs showing LOH in the tumor versus matched normal samples, this study identified three candidate genes with LOH (*MICU1*, *MCU* and *OIT3*) on chromosome 10 in pancreatic cancer, implying an important role for these genes in pancreatic cancer.

MCU and *MICU1* are two genes that have been demonstrated to play important roles in mitochondrial Ca^{2+} uptake (Perocchi et al., 2010; Baughman et al., 2011; Csordas et al., 2013). The Ca^{2+} handling by mitochondria is involved in cell life by triggering or preventing apoptosis probably functioning through the released pro-apoptotic factors such as Bax and Bak from the intermembrane space (Scorrano et al., 2003; Kroemer et al., 2007; Contreras et al., 2010). Thus, it could be speculated that *MCU* and *MICU1* may be related to the cancer progression via affecting cellular apoptosis. As expected, previous study has reported the down-regulation of *MCU* targeted by cancer-related microRNA may increase cancer cell survival and contribute to tumorigenesis in various cancer cells (Marchi et al., 2013). Silencing *MICU1* is also revealed to initiate the mitochondrial pathway for apoptosis by decreasing Bcl-2 expression together with increasing caspase-3 activity and cytosolic cytochrome c contents (Arvizo et al., 2013). Therefore, *MCU* and *MICU1* may be oncogene or tumor suppressor gene and the LOH in them may lead to their lower expression, thus preventing or promoting pancreatic cancer cell apoptosis.

OIT3 located at 10q22.1 was another gene with LOH found in pancreatic cancer. This gene, also termed as liver-specific zona pellucida domain-containing protein (LZP), is related to hepatocellular function and could be used as a potential diagnostic biomarker for hepatocellular carcinoma (Xu et al., 2003). Also, *OIT3* is reported to experience copy number losses and down-regulation in colorectal cancer (Yoshida et al., 2010). However, the details of the relationships between *OIT3* and cancers are not clear. Herein, *OIT3* was found to be associated with calcium ion binding, probably implying an involvement of this gene in cellular Ca^{2+} homeostasis. Moreover, based on the regulatory network in this study, *OIT3* was regulated by a large number of transcription factors including *STAT*, *SOX9*, *CREB*, *NF-kB*, *PPARG* and *p53* which were previously reported to be associated with pancreatic cancer. *STAT-3*, *PPARG* and *NF-kB* signaling pathways are involved in apoptosis and cellular differentiation in pancreatic cancer cells (Elnemr et al., 2000; Sahu and Srivastava, 2009). *CREB* is related to activate *STAT-3* and cyclin D1 expression in pancreatic cancer cells which lead to cell proliferation and tumor progression (Zhang et al., 2010). *SOX9* could initiate and accelerate the formation of premalignant lesions of pancreatic cancer (Kopp et al., 2012). In addition, mutations in *p53* tumor suppressor gene are considered to drive metastasis and contribute to the carcinogenesis of pancreatic cancer (Sato et al., 1996; Amaya et al., 2004; Morton et al., 2010). The LOH of *OIT3* may suggest dysregulated functions of these transcription factors, which may contribute to the progression of pancreatic cancer.

In conclusion, our global genomic analysis of SNPs provides evidence of LOH in *MICU1*, *MCU* and *OIT3* on chromosome 10 in pancreatic cancer. They may be involved in pancreatic cancer progression by regulating the calcium ion homogenizes and cell apoptosis. However, future mechanistic researches will be required to determine the molecular mechanisms.

Acknowledgements

We wish to express our warm thanks to Fenghe(Shanghai) Information Technology Co., Ltd. Their ideas and help gave a valuable added dimension to our research.

References

Amaya K, Ohta T, Kitagawa H, et al (2004). Angiotensin II activates MAP kinase and *NF-kB* through angiotensin II type I receptor in human pancreatic cancer cells. *Int J Oncol*, **25**, 849-56.

Arvizo RR, Moyano DF, Saha S, et al (2013). Probing novel roles of the mitochondrial uniporter in ovarian cancer cells using nanoparticles. *J Biol Chem*, **288**, 17610-8.

Baker DJ, Jin F, Jeganathan KB, et al (2009). Whole chromosome instability caused by Bub1 insufficiency drives tumorigenesis through tumor suppressor gene loss of heterozygosity. *Cancer cell*, **16**, 475-86.

Baughman JM, Perocchi F, Girgis HS, et al (2011). Integrative genomics identifies *MCU* as an essential component of the mitochondrial calcium uniporter. *Nature*, **476**, 341-5.

Beroukhim R, Lin M, Park Y, et al (2006). Inferring loss-of-heterozygosity from unpaired tumors using high-density oligonucleotide SNP arrays. *PLoS Comput Biol*, **2**, 41.

Bick AG, Calvo SE, Mootha VK (2012). Evolutionary diversity of the mitochondrial calcium uniporter. *Science*, **336**, 886-.

Butz J, Wickstrom E, Edwards J (2003). Characterization of mutations and loss of heterozygosity of *p53* and K-ras2 in pancreatic cancer cell lines by immobilized polymerase chain reaction. *BMC Biotechnol*, **3**, 11.

Chen K, Hsu L-T, Wu C-Y, et al (2013). CBARA1 plays a role in stemness and proliferation of human embryonic stem cells. *PloS One*, **8**, 63653.

Contreras L, Drago I, Zampese E, et al (2010). Mitochondria: the calcium connection. *BBA-Gen Subjects*, **1797**, 607-18.

Csordas G, Golenar T, Seifert EL, et al (2013). *MICU1* controls both the threshold and cooperative activation of the mitochondrial Ca^{2+} uniporter. *Cell Metab*, **17**, 976-87.

Csordas G, Varnai P, Golenar T, et al (2012). Calcium transport across the inner mitochondrial membrane: molecular mechanisms and pharmacology. Mol Cell Endocrinol, 353, 109-13.

Curry MC, Peters AA, Kenny PA, et al (2013). Mitochondrial calcium uniporter silencing potentiates caspase-independent cell death in MDA-MB-231 breast cancer cells. *Biochem Biophys Res Commun*, **434**, 695-700.

Day IN (2010). dbSNP in the detail and copy number complexities. *Hum Mutat*, **31**, 2-4.

Donahue TR, Tran LM, Hill R, et al (2012). Integrative survival-based molecular profiling of human pancreatic cancer. *Clin Cancer Res*, **18**, 1352-63.

Elnemr A, Ohta T, Iwata K, et al (2000). PPARgamma ligand (thiazolidinedione) induces growth arrest and differentiation markers of human pancreatic cancer cells. *Int J Oncol*, **17**, 1157-221.

Frampton SJ, King EV (2013). Loss of heterozygosity. in 'encyclopedia of otolaryngology, head and neck surgery',

Eds Springer, **1488**

Franko J, Krasinskas AM, Nikiforova MN, et al (2008). Loss of heterozygosity predicts poor survival after resection of pancreatic adenocarcinoma. *J Gastrointest Surg*, **12**, 1664-73.

Fujita PA, Rhead B, Zweig AS, et al (2010). The UCSC genome browser database: update 2011. *Nucleic Acids Res*, **963**.

Hoffman NE, Chandramoorthy HC, Shamugapriya S, et al (2013). *MICU1* motifs define mitochondrial calcium uniporter binding and activity. *Plant Cell Rep*, **5**, 1576-88.

Kohl M, Wiese S, Warscheid B (2011). Cytoscape: software for visualization and analysis of biological networks. , Eds Springer, **696**, 291-303

Kopp JL, von Figura G, Mayes E, et al (2012). Identification of Sox9-dependent acinar-to-ductal reprogramming as the principal mechanism for initiation of pancreatic ductal adenocarcinoma. *Cancer Cell*, **22**, 737-50.

Kroemer G, Galluzzi L, Brenner C (2007). Mitochondrial membrane permeabilization in cell death. *Physiol Rev*, **87**, 99-163.

Lin L-J, Asaoka Y, Tada M, et al (2008). Integrated analysis of copy number alterations and loss of heterozygosity in human pancreatic cancer using a high-resolution, single nucleotide polymorphism array. *Oncol*, **75**, 102-12.

Logan CV, Szabadkai G, Sharpe JA, et al (2014). Loss-of-function mutations in *MICU1* cause a brain and muscle disorder linked to primary alterations in mitochondrial calcium signaling. *Nat Genet*, **46**, 188-93.

Lucas AL, Shakya R, Lipsyc MD, et al (2013). High prevalence of *BRCA1* and *BRCA2* germline mutations with loss of heterozygosity in a series of resected pancreatic adenocarcinoma and other neoplastic lesions. *Clin Cancer Res*, **19**, 3396-403.

Mallilankaraman K, Doonan P, Cardenas C, et al (2012). *MICU1* Is an essential gatekeeper for *MCU*-mediated mitochondrial Ca< sup> 2+</sup> uptake that regulates cell survival. *Cell*, **151**, 630-44.

Marchi S, Lupini L, Patergnani S, et al (2013). Downregulation of the mitochondrial calcium uniporter by cancer-related miR-25. *Curr Biol*, **23**, 58-63.

Morton JP, Timpson P, Karim SA, et al (2010). Mutant *p53* drives metastasis and overcomes growth arrest/senescence in pancreatic cancer. *Proc Natl Acad Sci*, **107**, 246-51.

Patron M, Raffaello A, Granatiero V, et al (2013). The mitochondrial calcium uniporter (*MCU*): molecular identity and physiological roles. *J Biol Chem*, **288**, 10750-8.

Perocchi F, Gohil VM, Girgis HS, et al (2010). *MICU1* encodes a mitochondrial EF hand protein required for Ca^{2+} uptake. *Nature*, **467**, 291-6.

Raffaello A, De Stefani D, Rizzuto R (2012). The mitochondrial Ca^{2+} uniporter. *Cell Calcium*, **52**, 16-21.

Sahu RP, Srivastava SK (2009). The role of *STAT-3* in the induction of apoptosis in pancreatic cancer cells by benzyl isothiocyanate. *J Natl Cancer Inst*, **101**, 176-93.

Sato Y, Nio Y, Song M, et al (1996). *p53* protein expression as prognostic factor in human pancreatic cancer. *Anticancer Res*, **17**, 2779-88.

Scorrano L, Oakes SA, Opferman JT, et al (2003). BAX and BAK regulation of endoplasmic reticulum Ca^{2+}: a control point for apoptosis. *Science*, **300**, 135-9.

Sherman BT, Huang dW, Tan Q, et al (2007). DAVID Knowledgebase: a gene-centered database integrating heterogeneous gene annotation resources to facilitate high-throughput gene functional analysis. *BMC Bioinformatics*, **8**, 426.

Staaf J, Lindgren D, Vallon-Christersson J, et al (2008). Segmentation-based detection of allelic imbalance and loss-of-heterozygosity in cancer cells using whole genome SNP arrays. *Genome Biol*, **9**, 136.

Xu ZG, Du JJ, Zhang X, et al (2003). A novel liver-specific zona pellucida domain containing protein that is expressed rarely in hepatocellular carcinoma. *Hepatology*, **38**, 735-44.

Yoshida T, Kobayashi T, Itoda M, et al (2010). Clinical omics analysis of colorectal cancer incorporating copy number aberrations and gene expression data. *Cancer Informatics*, **9**, 147.

Zhang Y, Bharadwaj U, Logsdon CD, et al (2010). ZIP4 regulates pancreatic cancer cell growth by activating IL-6/*STAT*3 pathway through zinc finger transcription factor *CREB*. *Clin Cancer Res*, **16**, 1423-30.