Expression of Maternal Embryonic Leucine Zipper Kinase (MELK) Correlates to Malignant Potentials in Hepatocellular Carcinoma

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Abstract. *Background/Aim:* Maternal embryonic leucine zipper kinase (MELK) is categorized as a member of AMP-activated protein kinase families. Various MELK-associated cellular and biological processes affect multiple stages of tumorigenesis. The aim of the present study was to clarify the relationship between MELK expression and hepatocellular carcinoma (HCC) clinicopathological features. *Materials and Methods:* In thirty conserved frozen primary HCC and non-HCC samples MELK mRNA expression was examined by quantitative real-time polymerase chain reaction (PCR). *Results:* HCC tissues exhibited significantly higher expression levels compared to non-cancerous tissues. MELK expression had a statistically parallel correlation between tumor diameter and protein induced by vitamin K absence or antagonist II (PIVKA-II). The overall survival (OS) and recurrence-free survival (RFS) of the low MELK mRNA expression group was significantly longer than that of the high MELK mRNA expression group. *Conclusion:* MELK expression in HCC is extremely intense compared to its expression reported in other types of cancer. MELK could be a promising effective tumor marker of HCC and further consideration is needed.

Hepatocellular carcinoma (HCC) is the most common type of liver cancer. Currently, HCC is the third most deadly and fifth

most common cancer worldwide (1, 2). Chronic infection with hepatitis B virus (HBV), which affects approximately 5% of the global population, or hepatitis C virus (HCV), affecting approximately 2% of the global population, are risk factors for the development of HCC (3). A large number of patients are infected with hepatitis viruses in Japan and HCC is the fourth most deadly and sixth most common cancer according to Japanese vital statistics conducted in 2008 (4).

Maternal embryonic leucine zipper kinase (MELK) is categorized as a member of both the sucrose-non-fermenting (snf1) and the AMP-activated protein kinase (AMPK) families. This is because of the presence of a conserved serine/threonine kinase domain in the N-terminal region also known as murine protein serine-threonine kinase 38 (MPK38) (5) or Eg3 protein (6). Unlike other snf1/AMPK family members, MELK is activated by *in vitro* autophosphorylation (7). Various MELK-associated cellular and biological processes, including cell cycle, cell proliferation, apoptosis, gene expression, hematopoiesis and oncogenesis are involved in multiple protein interactions that affect multiple stages of tumorigenesis (8).

There is a wide expression pattern of MELK in human adult tissues, including the colon, esophagus, lymphoid tissue, skin, small intestine, soft tissue, testis, thymus and spleen (9). However, MELK is not present in the central nervous system, muscles, kidneys or liver (9). Regarding the relationship between cancer and MELK, there was a report that MELK mRNA levels were elevated in colon, breast, ovary and lung cancer samples compared to those in non-cancerous samples from the same organs. Additionally, knockdown of MELK decreased proliferation and anchorage-independent growth *in vitro*, as well as tumor growth in a xenograft model (9). Some studies have demonstrated a correlation between MELK

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expression and tumor malignancy grade for astrocytoma (10), breast cancer (11) and prostate cancer (12), as well as radiation and chemoresistance in colorectal cancer (13).

Therefore, the objective of this study was to clarify the relationship between MELK expression and HCC clinicopathological features.

Materials and Methods

Patients and study groups. We studied thirty conserved frozen primary HCC and non-HCC samples that were obtained *via* hepatic resection from 2008 to 2014 and pathologically diagnosed in our Department. Thirty paired samples that did not contain degraded RNA were used in this study. A group of thirty patients (23 men and seven women, average age=67.3 years) were included in the present study.

This study was approved by the Institutional Review Board of Graduate School of Medical and Dental Sciences Kagoshima University, Japan, and conducted according to the ethical guidelines of the Declaration of Helsinki. Written informed consent was given by each patient.

Clinicopathological factors. Clinicopathological factors selected for evaluation included fatty liver, diabetes mellitus, alcohol consumption and preoperative laboratory values (such as indocyanine green (ICG) retention rate at 15 min (ICGR15) value and tumor markers, including α -fetoprotein (AFP) and protein induced by vitamin K absence or antagonists-II (PIVKA-II) levels). A diagnosis of diabetes mellitus was based on the results of a 75-g oral glucose tolerance test or a random blood glucose measurement >200 mg/dl. Excessive alcohol consumption was defined as an average daily consumption of an amount equivalent to 80 g of pure ethanol for more than 10 years. Diabetes mellitus patients enrolled in the study included patients whose symptoms were medically well-controlled. Histopathological diagnosis was based on the evaluation of tumor size, number of tumor nodules, lymph node metastasis and infiltration of blood vessels (portal vein, hepatic artery and/or vein). HCC staging was conducted using The General Rules for the Clinical and Pathological Study of Primary Liver Cancer (14).

Cells and cell culture. Hepatoma cell lines HepG2, HuH7 and Li7 were provided by the Cell Resource Center for Biomedical Research Institute of Development, Aging, and Cancer at Tohoku University (Sendai, Japan). HepG2 were cultured with DMEM and HuH7, and Li7 were cultured with RPMI.

Quantitative real-time polymerase chain reaction (PCR). For reverse-transcription PCR and quantitative real-time PCR, total RNA was extracted from 30 mg frozen tissues using a phenol extraction method. For cDNA synthesis, the RNA samples (1 μ g) were converted into cDNA by reverse transcription using random primers (TAKARA, Siga, Japan) according to the manufacturer's instructions. To estimate the mRNA expression levels of several genes quantitatively, PCR amplification was performed using a Light-Cycler system (Roche, Mannheim, Germany) and the SYBER select master mix (Waltham, MA, USA). Primers were as follows: *MELK*: 5'-GCC TGC CAT ATC CTT ACT GG-3', 5'-TGG CTG TCT CTA GCA CAT GG-3', *HPRT*: 5'-GAC CAG TCA ACA GGG GAC AT-3', 5'-CTG CAT TGT TTT GCC AGT GT-3'. Reaction conditions were performed at an initial incubation at 95°C for 30 s,

followed by 40 cycles at 95°C for 5 s for denaturation, 60°C for 30 s for annealing of the primers and 54°C for 10 s for annealing and extension. Melting curves were obtained according to the protocol under the following conditions: 0 s denaturation period at 95°C, starting temperature of 65°C, end temperatures of 95°C and a rate of temperatures increase of 0.1°C/s. The quantitative value of the target gene (*MELK* mRNA) in each sample was normalized using hypoxanthine-guanine phosphoribosyltransferase (*HPRT*) expression as an internal control. The quantitative real time-PCR assay was performed twice and the mean value was calculated. Finally, the mRNA expression ratio of cancerous (C) to non-cancerous (N) tissues was calculated using the following formula: $R = \log\{MELK (C)/HPRT (C)\}$, $R = \log\{MELK (N)/HPRT (N)\}$. All experiments were performed twice to confirm reproducibility.

Statistical analyses. An unpaired t-test was used to evaluate continuous variables. The overall survival (OS) was calculated from the date of resection to the date of death regardless of the cause of death. The recurrence-free survival (RFS) was calculated from the date of resection to the date that the tumor recurrence was diagnosed or from the date of the resection to the last visit if the recurrence was not diagnosed. The cumulative OS and RFS rates were calculated using the Kaplan-Meier method and tested using the log-rank test. Data are presented as a mean $\pm$ standard deviation. A probability (*p*) value of <0.05 was considered to be statistically significant. Statistical analyzes were performed using the SPSS statistical software package (version 23; SPSS Inc., Chicago, IL, USA).

Results

Comparison of MELK mRNA expression between cancerous samples, non-cancerous samples, and HCC cell lines. Using real-time PCR, *MELK* and *HPRT* mRNA expression levels were examined between the three HCC cell lines (Huh7, HepG2 and Li7), as well as cancerous and non-cancerous tissues from two patients (Figure 1). Three cell lines and two cancer tissues highly expressed *MELK* mRNA. However, *MELK* mRNA expression was lower in the two non-cancerous tissues.

Quantitative MELK mRNA expression in HCC and non-cancerous lesions. Comparing the quantitative expression of *MELK* mRNA in paired cancer and non-cancerous tissues from 30 cases, HCC tissues exhibited significantly higher expression levels compared to non-cancerous tissues ($p < 0.001$, Figure 2).

Relationship between MELK mRNA expression and clinicopathological features. To elucidate the biological significance of MELK expression in HCC, we compared the levels of *MELK* mRNA expression with the clinicopathological features of 30 patients. *MELK* mRNA expression was significantly higher in the tumor size ≥ 3.75 cm ($p = 0.015$; Table I), the PIVKA II ≥ 40 mAU/ml ($p = 0.047$; Table I), the advanced tumor stages ($p = 0.005$; Table I) and had a tendency to be lower in simple nodular types of tumors ($p = 0.088$, Table I) within the pathological parameter.

Figure 1. *MELK* and *HPRT* mRNA expression levels were examined between the three HCC cell lines (Huh7, HepG2 and Li7), as well as cancerous and non-cancerous tissues from two patients. Three cell lines and two cancer tissues highly expressed *MELK* mRNA. However, *MELK* mRNA expression was lower in the two non-cancerous tissues.

Comparison of OS and RFS between MELK mRNA high and low groups. We further investigated the correlation between the relative *MELK* mRNA levels, as well as the OS and RFS of patients with HCC. When we set a cut-off value of 0.7 in this examination, OS (median survival=65.5 months vs. 34.9 months; $p=0.019$; Figure 3) and RFS (median survival=42.3 months vs. 11.0 months; $p=0.012$; Figure 4) of the low *MELK* mRNA expression group was significantly longer than that of the high *MELK* mRNA expression group.

Discussion

MELK was identified in mice using differential display analyses of cDNA libraries from unfertilized eggs and preimplantation embryos (15). *MELK* is expressed across a wide range of embryonic stages and the gene has been shown to play an important role in preimplantation embryonic development (16).

MELK was reported to bind to c-Jun (17), p53 (18), tumor necrosis receptor-associated factor (19), Daxx (20), thioredoxin (21, 22), glutaredoxin (23), heat shock protein

72 (24), Raf-1 (21), FOXM1 (25) and Akt/PKB (26). In particular, c-Jun is a family of MAP kinases, including the c-Jun NH(2)-terminal kinases (JNKs). The JNK pathway induces cell proliferation and apoptosis. Tumor-specific *MELK* interaction with c-Jun can contribute to cellular proliferation and selective apoptosis (27). Moreover, *MELK* associated with p53 could enhance p53-mediated cell cycle arrest and apoptosis (18).

Concerning the cell cycle, *MELK*-regulated phosphorylation of FOXM1, a master regulator of cell-cycle progression (27), facilitated *FOXM1* transcriptional activity. Moreover, *MELK* expression was similar to that of cyclin A, cyclin B and CDK1 and 4 for mitotic progression (28, 29) and was co-regulated with mitosis-phase regulatory protein ASPM and Aurora kinase B (30). With respect to the regulation of apoptosis, *MELK* was associated with resistance to apoptosis through inhibition of Bcl-G (31) and its regulation of p53 activity (32).

There have been some reports that *MELK* was expressed in cancer stem cells or initiating cells. High levels of *MELK* expression in mammary tumors resulted in a significant

Figure 2. Comparing the quantitative expression of MELK mRNA in paired cancer and non-cancerous tissues from 30 cases, HCC tissues exhibited significantly higher expression levels compared to non-cancerous tissues. Unpaired t-test.

Figure 3. At a cut-off value of 0.7, RFS (median survival=42.3 months vs. 11.0 months) of the low MELK mRNA expression group was significantly longer than that of the high MELK mRNA expression group. Kaplan-Meier method, tested using the log-rank test.

enrichment of tumorsphere formation in culture and tumor initiation after transplantation (33). In addition, the transcription factor and oncogene FOXM1 formed a protein complex with MELK. Moreover, the transgenic expression of FOXM1 enhanced neurosphere formation using mouse neural progenitor cells and suggested that FOXM1-MELK binding

Figure 4. At a cut-off value of 0.7, OS (median survival=65.5 months vs. 34.9 months) of the low MELK mRNA expression group was significantly longer than that of the high MELK mRNA expression group. Kaplan-Meier method, tested using the log-rank test.

was required for neural progenitor cell growth (25). Therefore, MELK is associated with the basis of cell proliferation, including cancer progression.

Regarding the relationship between human cancers and MELK, Pickard *et al.* reported that MELK controlled apoptosis through Fau, a novel apoptosis regulator. Additionally, MELK expression was elevated in breast cancer tissue and this increase was also associated with poor patient survival (11). Similarly, Wang *et al.* reported that increased levels of MELK expression was detected in particularly aggressive subtypes of breast cancer (*e.g.*, basal-like breast cancer) and correlated with poor prognosis (34). Du *et al.* reported MELK-associated cell cycle and elevated expression levels in human gastric cancer and found associated with chemoresistance (35). Marie *et al.* found progressively higher MELK expression associated with the astrocytoma grade. This study also revealed a noteworthy uniformity of high levels of expression in glioblastoma multiforme. The knockdown of MELK in malignant astrocytoma cell lines resulted in a reduction in proliferation and anchorage-independent growth as demonstrated by *in vitro* assays (10).

These reports coincide with our results that HCC tissues exhibited significantly higher expression levels compared to non-cancerous tissues ($p < 0.001$, Figure 2) and MELK mRNA expression was significantly higher in advanced tumor stages ($p = 0.005$; Table I) and had a tendency to be

Table I. *MELK* mRNA expression and clinicopathological features.

Variable		<i>MELK</i> mRNA	<i>p</i> -Value
Tumor/non-tumor	0.436±0.437	0.019±0.023	<0.001
Fatty liver			
Yes (4)/No (26)	0.270±0.289	0.457±0.443	0.423
Diabetes mellitus			
Yes (7)/No (23)	0.602±0.564	0.387±0.384	0.247
Alcohol consumption			
Yes (5)/No (25)	0.462±0.203	0.429±0.461	0.878
ICGR15 ≥10%			
Yes (22)/No (8)	0.404±0.465	0.578±0.358	0.374
AFP ≥10 ng/ml			
Yes(20)/No(10)	0.433±0.433	0.437±0.441	0.980
PIVKA II ≥ 40 mAU/ml			
Yes (21)/No (9)	0.539±0.487	0.196±0.093	0.047
Tumor size ≥3.75 cm			
Yes (15)/No (15)	0.625±0.462	0.246±0.323	0.015
Tumor numbers ≥2			
Yes (11)/No (19)	0.502±0.553	0.398±0.366	0.538
Tumor stage			
I, II (17)/III, IV (13)	0.624±0.478	0.290±0.207	0.005
Virus			
None (18)/B (4)/C (8)	0.523±0.458	0.753±0.528	0.386±0.478 0.815
Pathological parameter			
Macroscopical type;			
Simple nodular type			
Yes (13)/No (17)	0.280±0.292	0.555±0.498	0.088
Microscopical type;			
Well (6)/moderately (23)/poor(1)	0.470±0.415	0.483±0.571	0.335±0.469 0.778
Infiltration to blood vessel			
Yes (14)/No (16)	0.555±0.444	0.332±0.418	0.167

Data are presented as number, percent and median±standard deviation, tested using the unpaired *t*-test. AFP, α -Fetoprotein; ICGR15, indocyanine green retention rate at 15 min; PIVKA II, protein induced by vitamin K absence or antagonist II. Pathological parameter is classified by the General Rules for the Clinical and Pathological Study of Primary Liver Cancer the 6th Edition (Liver Cancer Study Group of Japan).

lower for simple nodular types ($p=0.088$; Table I), which was the less aggressive type of the pathological parameters. The OS and RFS of the low *MELK* mRNA expression group were significantly longer than that of the high *MELK* mRNA expression group (Figures 3 and 4). *MELK* expression correlates to malignant potential and this study is the first report that examined *MELK* expression in HCC.

Conclusion

In summary, *MELK* is up-regulated in various types of cancer and our data also show *MELK* expression in HCC is up-regulated. Furthermore, *MELK* expression in HCC is extremely intense compared to previously reported types of cancer as *MELK* is involved in multiple protein interactions affecting many situations of tumorigenesis and cancer initiation. Therefore, *MELK* could be a promising effective tumor marker and a therapeutic target of HCC that could be used clinically in the near future.

References

- 1 El-Serag HB and Mason AC: Rising incidence of hepatocellular carcinoma in the United States. *N Engl J Med* 340: 745-750, 1999.
- 2 Bosch FX, Ribes J and Borrás J: Epidemiology of primary liver cancer. *Semin Liver Dis* 19: 271-285, 1999.
- 3 El-Serag HB: Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 142: 1264-1273, 2012.
- 4 Japan Ministry of Health, Labor and Welfare carried out in 2008
- 5 Gil M, Yang Y, Lee Y, Choi I and Ha H: Cloning and expression of a cDNA encoding a novel protein serine/threonine kinase predominantly expressed in hematopoietic cells. *Gene* 195: 295-301, 1997.
- 6 Blot J, Chartrain I, Roghi C, Philippe M and Tassan JP: Cell cycle regulation of pEg3, a new *Xenopus* protein kinase of the KIN1/PAR-1/MARK family. *Dev Biol* 241: 327-338, 2002.
- 7 Lizcano JM, Göransson O, Toth R, Deak M, Morrice NA, Boudeau J, Hawley SA, Udd L, Mäkelä TP, Hardie DG and Alessi DR: LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. *EMBO J* 23: 833-843, 2004.
- 8 Chung S, Suzuki H, Miyamoto T, Takamatsu N, Tatsuguchi A, Ueda K, Kijima K, Nakamura Y and Matsuo Y: Development of an orally-

- administrative MELK-targeting inhibitor that suppresses the growth of various types of human cancer. *Oncotarget* 3: 1629-1640, 2012.
- 9 Gray D, Jubb AM, Hogue D, Dowd P, Kljavin N, Yi S, Bai W, Frantz G, Zhang Z, Koeppen H, de Sauvage FJ and Davis DP: Maternal embryonic leucine zipper kinase/murine protein serine-threonine kinase 38 is a promising therapeutic target for multiple cancers. *Cancer Res* 65: 9751-9761, 2005.
- 10 Marie SK, Okamoto OK, Uno M, Hasegawa AP, Oba-Shinjo SM, Cohen T, Camargo AA, Kosoy A, Carlotti CG Jr, Toledo S, Moreira-Filho CA, Zago MA, Simpson AJ and Caballero OL: Maternal embryonic leucine zipper kinase transcript abundance correlates with malignancy grade in human astrocytomas. *Int J Cancer* 122: 807-815, 2008.
- 11 Pickard MR, Green AR, Ellis IO, Caldas C, Hedge VL, Mourtada-Maarabouni M and Williams GT: Dysregulated expression of Fau and MELK is associated with poor prognosis in breast cancer. *Breast Cancer Res* 11(4): R60, 2009.
- 12 Kuner R, Falth M, Pressinotti NC, Brase JC, Puig SB, Metzger J, Gade S, Schafer G, Bartsch G, Steiner E, Klocker H and Sultmann H: The maternal embryonic leucine zipper kinase (MELK) is upregulated in high-grade prostate cancer. *J Mol Med (Berl)* 91(2): 237-248, 2013.
- 13 Choi S and Ku JL: Resistance of colorectal cancer cells to radiation and 5-FU is associated with MELK expression. *Biochem Biophys Res Commun* 412(2): 207-213, 2011.
- 14 Liver Cancer Study Group of Japan. The General Rules for the Clinical and Pathological Study of Primary Liver Cancer
- 15 Heyer BS, Warsowe J, Solter D, Knowles BB and Ackerman SL: New member of the Snf1/AMPK kinase family, Melk, is expressed in the mouse egg and preimplantation embryo. *Mol Reprod Dev* 47: 148-156, 1997.
- 16 Heyer BS, Kochanowski H and Solter D: Expression of Melk, a new protein kinase, during early mouse development. *Dev Dyn* 215: 344-351, 1999.
- 17 Gu C, Banasavadi-Siddegowda YK, Joshi K, Nakamura Y, Kurt H, Gupta S and Nakano I: Tumor-specific activation of the C-JUN/MELK pathway regulates glioma stem cell growth in a p53-dependent manner. *Stem Cells* 31: 870-881, 2013.
- 18 Seong HA and Ha H.: Murine protein serine-threonine kinase 38 activates p53 function through Ser15 phosphorylation. *J Biol Chem* 287: 20797-20810, 2012.
- 19 Nishitoh H, Saitoh M, Mochida Y, Takeda K, Nakano H, Rothe M, Miyazono K, Ichijo H, Imamura H and Sano K: ASK1 is essential for JNK/SAPK activation by TRAF2. *Mol Cell* 2: 389-395, 1998.
- 20 Chang HY, Nishitoh H, Yang X, Ichijo H and Baltimore D: Activation of apoptosis signal-regulating kinase 1 (ASK1) by the adapter protein Daxx. *Science* 281: 1860-1863, 1998.
- 21 Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata M, Miyazono K and Ichijo H: Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO J* 17: 2596-2606, 1998.
- 22 Liu H, Nishitoh H, Ichijo H and Kyriakis JM: Activation of apoptosis signal-regulating kinase 1 (ASK1) by tumor necrosis factor receptor-associated factor 2 requires prior dissociation of the ASK1 inhibitor thioredoxin. *Mol Cell Biol* 20: 2198-2208, 2000.
- 23 Song JJ, Rhee JG, Suntharalingam M, Walsh SA, Spitz DR and Lee YJ: Role of glutaredoxin in metabolic oxidative stress. Glutaredoxin as a sensor of oxidative stress mediated by H₂O₂. *J Biol Chem* 277: 46566-46575, 2002.
- 24 Park HS1, Cho SG, Kim CK, Hwang HS, Noh KT, Kim MS, Huh SH, Kim MJ, Ryoo K, Kim EK, Kang WJ, Lee JS, Seo JS, Ko YG, Kim S and Choi EJ: Heat shock protein hsp72 is a negative regulator of apoptosis signal-regulating kinase 1. *Mol Cell Biol* 22: 7721-7730, 2002.
- 25 Joshi K, Banasavadi-Siddegowda Y, Mo X, Kim SH, Mao P, Kig C, Nardini D, Sobol RW, Chow LM, Kornblum HI, Waclaw R, Beullens M and Nakano I: MELK-dependent FOXM1 phosphorylation is essential for proliferation of glioma stem cells. *Stem Cells* 31: 1051-1063, 2013.
- 26 Kim AH, Khursigara G, Sun X, Franke TF and Chao MV: Akt phosphorylates and negatively regulates apoptosis signal-regulating kinase 1. *Mol Cell Biol* 21: 893-901, 2001.
- 27 Gu C, Banasavadi-Siddegowda YK, Joshi K, Nakamura Y, Kurt H, Gupta S and Nakano I: Tumor-specific activation of the C-JUN/MELK pathway regulates glioma stem cell growth in a p53-dependent manner. *Stem Cells* 31: 870-881, 2013.
- 28 Badouel C, Chartrain I, Blot J and Tassan JP: Maternal embryonic leucine zipper kinase is stabilized in mitosis by phosphorylation and is partially degraded upon mitotic exit. *Exp Cell Res* 316: 2166-2173, 2010.
- 29 Nakano I, Paucar AA, Bajpai R, Dougherty JD, Zewail A, Kelly TK, Kim KJ, Ou J, Groszer M, Imura T, Freije WA, Nelson SF, Sofroniew MV, Wu H, Liu X, Terskikh AV, Geschwind DH and Kornblum HI: Maternal embryonic leucine zipper kinase (MELK) regulates multipotent neural progenitor proliferation. *J Cell Biol* 170: 413-427, 2005.
- 30 Horvath S1, Zhang B, Carlson M, Lu KV, Zhu S, Felciano RM, Laurance MF, Zhao W, Qi S, Chen Z, Lee Y, Scheck AC, Liao LM, Wu H, Geschwind DH, Febbo PG, Kornblum HI, Cloughesy TF, Nelson SF and Mischel PS: Analysis of oncogenic signaling networks in glioblastoma identifies ASPM as a molecular target. *Proc Natl Acad Sci USA* 103: 17402-17407, 2006.
- 31 Lin ML, Park JH, Nishidate T, Nakamura Y and Katagiri T: Involvement of maternal embryonic leucine zipper kinase (MELK) in mammary carcinogenesis through interaction with Bcl-G, a pro-apoptotic member of the Bcl-2 family. *Breast Cancer Res* 9: R17, 2007.
- 32 Ganguly R, Mohyeldin A, Thiel J, Kornblum HI, Beullens M and Nakano I: MELK-a conserved kinase: Functions, signaling, cancer, and controversy. *Clin Transl Med* doi: 10.1186/s40169-014-0045-y, 2015.
- 33 Hebbard LW, Maurer J, Miller A, Lesperance J, Hassel J, Oshima RG and Terskikh AV: Maternal embryonic leucine zipper kinase is upregulated and required in mammary tumor-initiating cells *in vivo*. *Cancer Res* 70: 8863-8873, 2010.
- 34 Wang Y, Lee YM, Baitsch L, Huang A, Xiang Y, Tong H, Lako A, Von T, Choi C, Lim E, Min J, Li L, Stegmeier F, Schlegel R, Eck MJ, Gray NS, Mitchison TJ and Zhao JJ: MELK is an oncogenic kinase essential for mitotic progression in basal-like breast cancer cells. *Elife* doi: 10.7554/eLife.01763, 2014.
- 35 Du T, Qu Y, Li J, Li H, Su L, Zhou Q, Yan M, Li C, Zhu Z and Liu B: Maternal embryonic leucine zipper kinase enhances gastric cancer progression *via* the FAK/Paxillin pathway. *Mol Cancer* doi: 10.1186/1476-4598-13-100, 2014.

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