

RESEARCH ARTICLE

Overexpression of TRPM7 is Associated with Poor Prognosis in Human Ovarian Carcinoma

Jing Wang^{1,2}, Ling Xiao³, Chen-Hui Luo², Hui Zhou², Jun Hu², Yu-Xi Tang², Kai-Ning Fang², Yi Zhang^{1*}

Abstract

Background: The melastatin-related transient receptor potential 7 channel (TRPM7) is a nonselective cation channel that has been shown to promote tumor metastasis and progression. In this study, we determined the expression of TRPM7 in ovarian carcinomas and investigated its possible prognostic value. **Materials and Methods:** Samples were collected from 138 patients with ovarian cancer. Expression of TRPM7 was assessed by real-time PCR and immunohistochemistry, expressed with reference to an established scoring system and related to clinical pathological factors. Kaplan-Meier survival analysis was applied to estimate disease-free survival (DFS) and overall survival (OS). Univariate and multivariate cox regression analyses were performed to correlate TRPM7 expression levels with DFS and OS. **Results:** TRPM7 was highly expressed in ovarian carcinoma and significantly associated with decreased disease-free survival (DFS: median 20 months vs. 42 months, $P=0.0002$) and overall survival (OS: median 27 months vs. 46 months, $P<0.001$). **Conclusion:** Overexpression of TRPM7 expression is significantly associated with poor prognosis in patients with ovarian cancer.

Keywords: Ovarian cancer - TRPM7 - ion channel - prognosis

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Introduction

Ovarian cancer is one of the most common cancer in women and it is the leading cause of death in gynaecological cancer (Siegel et al., 2013). In China, the age-adjusted incidence and motility rate of ovarian cancer is 5.35 and 1.6 per 100,000, respectively (Kim et al., 2009; Wang et al., 2014). Although substantial advances in surgical techniques and chemotherapeutic treatments have been made in ovarian cancer, only approximately 30% patients with ovarian cancer can survive 5 years after the initial diagnosis (Vaughan et al., 2011). The poor ratio of survival is primarily attribute to lacking of specific symptoms and effective methods for the early detection in ovarian cancer patients (Vaughan et al., 2011). In addition, the development of multidrug resistances also leads to the failure in treatment of ovarian cancer (Thigpen et al., 2011; Pitakkarnkul et al., 2013). Thus, identification of molecular markers for prognosis is important for improving therapeutic methods and extending survival of ovarian cancer patients.

The transient receptor potential (TRP) channels play a key role in maintaining cellular calcium homeostasis (Ramsey et al., 2006). They are also reported to express in cancer cells and be involved in tumor development

and progression (Prevarskaya et al., 2007). According to sequence homology, the TRP superfamily is divided into six subfamilies (Clapham, 2003). TRPM7 belongs to the melastatin transient receptor potential (TRPM) channel subfamily, which includes eight members, from TRPM1 to TRPM8 (Ramsey et al., 2006). TRPM7 contains both a cation-conducting pore and a serine-threonine kinase, which is a unique feature of cation channels fused with a kinase function (Paravicini et al., 2012). As a cation selective ion channel, TRPM7 is permeable to both Ca^{2+} and Mg^{2+} (Minke, 2006). TRPM7 widely expressed in multiple organs including heart, lung, liver, brain and spleen (Nadler et al., 2001).

Recently, elevate expression of TRPM7 was found in different types of cancers such as breast cancer, retinoblastoma, neck and head carcinoma, pancreatic adenocarcinoma and lung cancer (Hanano et al., 2004; Jiang et al., 2007; Dhennin-Duthille et al., 2011; Gao et al., 2011; Middelbeek et al., 2012; Rybarczyk et al., 2012). In cancer cells, TRPM7 has been reported to regulate cell proliferation, adhesion, migration and survival. For example, in breast cancer, overexpression of TRPM7 was associated with breast cancer progression and metastasis formation (Middelbeek et al., 2012). However, little is known about the expression and prognostic value of

¹Department of Obstetrics and Gynaecology, Xiangya Hospital, Central South University, ²The Affiliated Cancer Hospital of Xianaya School of Medicine, Central South University, ³Department of Histology and Embryology of School of Basic Medical Sciences, Central South University, Changsha, China *For correspondence: yizhang608@126.com

TRPM7 in ovarian cancer. In this study, we characterized the expression pattern and evaluated prognostic value of TRPM7 in patients with ovarian cancer.

Materials and Methods

Patients

A total of 138 ovarian carcinoma specimens were obtained from the patients diagnosed as primary ovarian cancer after surgery at Tumor Hospital of Xiangya School of Medicine from Jan.2005 to Dec. 2010. Cisplatin-based adjuvant chemotherapies following cytoreduction were applied to all the patients. Surgical staging was established according to the International Federation of Gynecology and Obstetrics (FIGO) system. Histopathological classification was performed by an experienced pathologist (Table 1). Disease-free survival (DFS) and Overall survival (OS) were analyzed as previously described (He et al., 2013). This study was approved by the Research Ethics Committee of Tumor Hospital of Xiangya School of Medicine, Hunan, and China. Informed consent was obtained from all of the patients.

Real-time PCR

The ovarian specimens were collected with patient consent. The solid tumor samples and adjacent normal ovarian tissues were dissected from patients. Total RNA was isolated with TRIzol reagent (Invitrogen). cDNA was prepared by oligo (dT)12–18 and reverse transcriptase SuperScript II from Invitrogen with 2 µg of DNase I-treated total RNA. TRPM7 specific primers (forward: TAGCCTTTAGCCACTGGAC; reverse: GCATCTTCTCCTAGATTTGC) was used for real-time PCR analysis. PCR was performed using SYBR® Green Real Time PCR Master Mixes (Invitrogen) and conducted with the MyiQ single color real time PCR detection system (Bio-Rad). Two sets of PCR assays were performed for each sample. The threshold cycle number for TRPM7 in each sample was normalized to that of GAPDH (primer forward: AGTCCCTGCCACACTCAGTC, reverse: GCA CAGGGTACTTTATTGATGG), and averaged. The average relative value for each sample was dot plotted on a graph.

Immunohistochemistry

Paraffin-embedded tissues were stained with anti-TRPM7 antibody (1:200 dilution, clone S74-25, Cayman Chemical Company, USA) at 4 °C overnight. Mouse IgG was used as negative control. After washing, the slides were treated with mouse biotinylated secondary antibody at room temperature for 30 min. And then, the slides were stained with the ABC Elite kit (Vectorlabs, CA). Finally, the slides were counterstained with hematoxylin, dehydrated, cleared and then mounted with Permount mounting medium (Fisher Scientific, PA). TRPM7 staining was scored independently by two pathologists and was calculated using a previously defined scoring system (Pham et al., 2013; Qiu et al., 2013). Briefly, the proportion of positive tumor cell was scored as: 0 = less than 5%; 1+ = 5%–20 %; 2+ = 21%–50 %; and 3+ >

50 %. The intensity was arbitrarily scored as 0 = weak (no color or light blue), 1 = moderate (light yellow), 2= strong (yellow brown) and 3 = very strong (brown). The overall score was calculated by multiplying the two scores obtained from each sample. A score of ≥ 4 was defined as high TRPM7 expression and scores of < 4 defined low TRPM7 expression.

Statistical analysis

All data were analyzed using the SPSS (version 20.0; IBM Corporation, Armonk, NY, USA) software program. The relationship between the expression of TRPM7 and clinic pathological factors was analyzed using the χ^2 test or Fisher's exact test, as appropriate. Survival curves were plotted by Kaplan–Meier method and compared by log-rank test. Univariate and multivariate analyses were performed using Cox regression models. *P* value less than 0.05 was regarded as statistically significant.

Results

To characterize the expression of TRPM7 in human ovarian cancer, we first performed real-time PCR to examine the mRNA of TRPM7 in 21 fresh human ovarian carcinomas. As shown in Figure 1A, the mRNA levels of TRPM7 were increased in 21 ovarian carcinomas compared with the paired normal ovarian tissues. We then performed immunohistochemistry to measure the protein

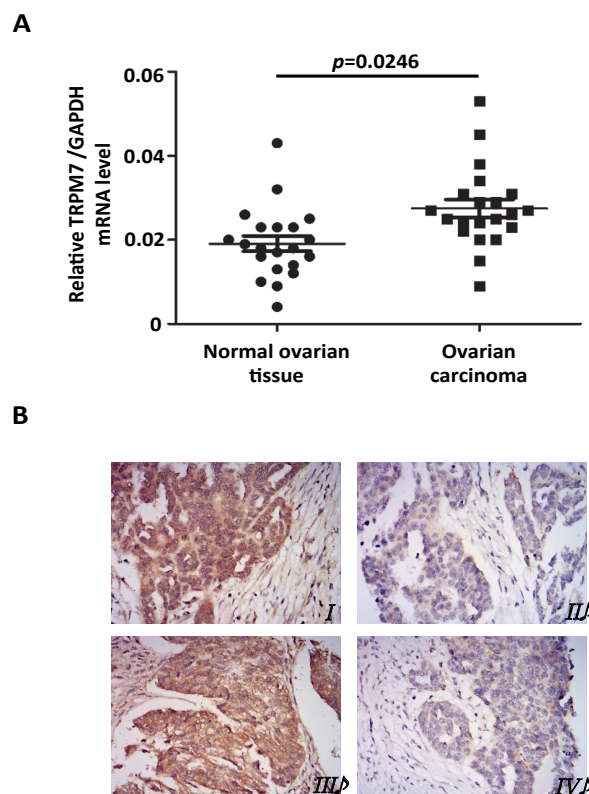


Figure 1. Expression of TRPM7 in Ovarian Carcinomas. (A) A dot plot of relative TRPM7 mRNA levels in normal ovarian or ovarian carcinomas. (B) Representative expression patterns of TRPM7 in ovarian carcinomas. I, Serous ovarian cancer tissue with high TRPM7 expression. II, Serous ovarian cancer tissue with low TRPM7 expression. III, Mucinous ovarian cancer tissue with high TRPM7 expression. IV, Mucinous ovarian cancer tissue with low TRPM7 expression. Magnification, X400

Table 1. Clinic Pathological Characteristics and Results of TRPM7 Immunohistochemistry

Characteristics	No. of patients	TRPM7 expression		P
		Low or no	High	
Ages				1
≤60	62	28	34	
>60	76	35	41	
Histologic type				0.562
Serous	70	29	41	
Mucinous	38	17	21	
Endometrioid	20	11	9	
Clear cell	10	6	4	
Pathological grade				0.2717
1	27	16	11	
2	50	22	28	
3	61	25	36	
FIGO stage				0.9167
I-II	62	28	34	
III-IV	76	35	41	
Pelvic metastasis				0.0005
-	81	47	34	
+	57	16	41	

Abbreviation, FIGO, International Federation of Gynecology and Obstetrics

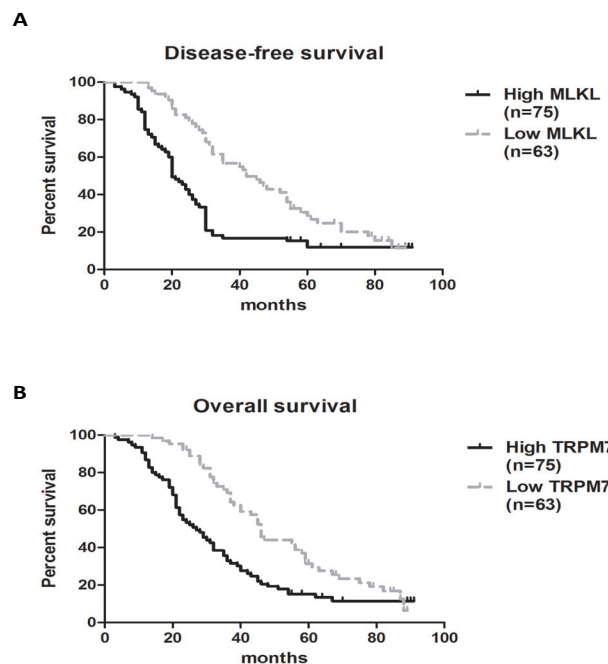


Figure 2. Kaplan-Meier Log-rank Survival Analysis for (A) Disease-free Survival and (B) Overall Survival of Ovarian Cancer Patients According to TRPM7 Expression. Abbreviation: n, number

levels of TRPM7 in 138 paraffin-embedded ovarian cancer tissues. TRPM7 was expressed in 96 (69.5%) of the 138 tumours evaluated, with diffuse cytoplasmic location of the immunoreaction (Figure 1B). These data suggest that TRPM7 is overexpressed in the examined ovarian cancer tissues.

To investigate the potential role of TRPM7 in progression of ovarian cancer, the association of TRPM7 expression with clinic pathological characteristics was analyzed in the patients. Patient characteristics of the population are summarized in Table 1. We found

Table 2. Multivariate Analyses for All Patients (n=138)

Characteristics	DFS		OS	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Ages (<=60 years vs. >60 years)	1.3 (0.8-2.0)	0.3681	1.4 (0.8-1.7)	0.9516
Histologic type (serous vs. mucinous vs. endometrioid vs. clear cell)	1.2 (0.6-1.6)	0.1926	0.9 (0.2-1.7)	0.5132
Pathological grade (grade 1 vs. grade 2 vs. grade 3)	1.1 (0.7-2.1)	0.3265	1.1 (0.8-1.4)	0.2317
FIGO stage (I-II vs. III-IV)	0.8 (0.6-1.8)	0.1731	0.7 (0.4-1.6)	0.0827
Pelvic metastasis (positive vs. negative)	2.8 (0.6-5.2)	0.0134	1.6 (0.8-4.7)	0.0159
Level of TRPM7 expression (high vs. low)	3.6 (0.5-6.1)	0.0082	3.8 (1.3-7.6)	0.0031

HR, hazard ratio; CI, confidence interval; DFS, disease-free survival; OS, overall survival

that TRPM7 protein expression was associated pelvic metastasis (Table 1). However, no statistical associations were observed between the expression of TRPM7 and patient age, histological type, pathologic grade or FIGO stage (Table 1).

Then, the prognostic significance of TRPM7 was evaluated in the patients. We found that high TRPM7 expression was significantly associated with poor disease-free survival (DFS: median 20 months vs. 42 months, $p=0.0002$) and shorter overall survival (OS: median 27 months vs. 46 months, $p<0.001$) (Figure 2A and B). Furthermore, a multivariate Cox regression analysis was applied to all of the clinic pathological characteristics with TRPM7 expression levels. As shown in Table 2, high TRPM7 expression levels were independently associated with the poor prognosis of patients with ovarian cancer.

Discussion

To our knowledge, this is first time to report that TRPM7 is highly expressed in ovarian cancer and is closely correlated with bad clinical prognosis in patients with ovarian cancer. Our results demonstrate that TRPM7 overexpressed in ovarian carcinomas and may represent an independent prognostic factor for the outcome in patients with ovarian cancer.

As a member of TRP protein family, TRPM7 is ubiquitously expressed and controls cellular homeostasis of ions, particularly Mg^{2+} and Ca^{2+} (Nadler et al., 2001). TRPM7 also modulates the signaling pathways involved in cell cycle progression, adhesion, survival, and migration. In cancer cells, TRPM7 has been shown to promote cell adhesion, proliferation and migration (Yee et al., 2012a). For example, TRPM7 was found overexpressed in grade III breast cancer samples and promote breast cancer proliferation and migration (Dhennin-Duthille et al., 2011; Middelbeek et al., 2012). In pancreatic adenocarcinoma, TRPM7 was found highly up-regulated in cancer tissues and knockdown TRPM7 by small interference RNA induced cellular senescence (Rybarczyk et al., 2012; Yee et al., 2012b). In lung cancer, TRPM7 expression was up-regulated by epidermal growth factor (EGF) and plays a pivotal role in the migration of A549 cells (Gao et al., 2011). Our real time RT-PCR experiment showed that

there was an up-regulation of TRPM7 mRNA in ovarian carcinoma tissues compared with normal ovarian tissues. Moreover, high TRPM7 expression was associated with pelvic metastasis of ovarian carcinoma. These results may suggest a positive role of TRPM7 in regulation cell proliferation and migration in ovarian cancer.

To further investigate the biological roles of TRPM7 in ovarian cancer, we then analyzed the correlation between TRPM7 expression and prognosis in patients with ovarian cancer. We identified that higher TRPM7 expression in ovarian cancer patients was correlated to worse DFS and OS. It has been reported that in the patients with breast or pancreatic cancer, overexpression of TRPM7 was significantly related with overall and disease-free survival (Middelbeek et al., 2012; Rybarczyk et al., 2012). Our data not only imply a potentially promising application of TRPM7 as a valuable prognostic marker, but suggest a possible relationship between the molecular functions of TRPM7 and the carcinogenesis of ovarian cancer. Further studies need to examine the association between TRPM7 and other prognostic biomarkers (Bian et al., 2013) and may identify a prognostic panel including multiple risk factors in ovarian cancer patients (Ma et al., 2012).

In conclusion, we explore the expression of TRPM7 in the context of ovarian cancer and demonstrate that high TRPM7 expression is associated with tumor progression and pelvic metastasis of ovarian cancer. This study suggests that TRPM7 may serve as a potential prognosis factor in patients with ovarian cancer. However, further studies are needed to clarify the mechanism by which TRPM7 is involved in the development and metastasis of ovarian cancer.

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References

Bian J, Li B, Kou XJ, et al (2013). Clinical significance of combined detection of serum tumor markers in diagnosis of patients with ovarian cancer. *Asian Pac J Cancer Prev*, **14**, 6241-3.

Clapham, DE. (2003). TRP channels as cellular sensors. *Nature*, **426**, 517-24.

Dhennin-Duthille I, Gautier M, Faouzi M, et al (2011). High expression of transient receptor potential channels in human breast cancer epithelial cells and tissues: correlation with pathological parameters. *Cell Physiol Biochem*, **28**, 813-22.

Gao H, Chen X, Du X, et al (2011). EGF enhances the migration of cancer cells by up-regulation of TRPM7. *Cell Calcium*, **50**, 559-68.

Hanano T, Hara Y, Shi J, et al (2004). Involvement of TRPM7 in cell growth as a spontaneously activated Ca²⁺ entry pathway in human retinoblastoma cells. *J Pharmacol Sci*, **95**, 403-19.

He L, Peng K, Liu Y, et al (2013). Low expression of mixed lineage kinase domain-like protein is associated with poor prognosis in ovarian cancer patients. *Onco Targets Ther*, **6**, 1539-43.

Jiang J, Li MH, Inoue K, et al (2007). Transient receptor potential melastatin 7-like current in human head and neck carcinoma

cells: role in cell proliferation. *Cancer Res*, **67**, 10929-38.

Kim K, Zang R, Choi SC, et al (2009). Current status of gynecological cancer in China. *J Gynecol Oncol*, **20**, 72-6.

Ma SH, Kim BG, Choi JY, et al (2012). Korean epithelial ovarian cancer study (Ko-EVE): protocols and interim report. *Asian Pac J Cancer Prev*, **13**, 3731-40.

Middelbeek J, Kuipers AJ, Henneman L, et al (2012). TRPM7 is required for breast tumor cell metastasis. *Cancer Res*, **72**, 4250-61.

Minke B. (2006). TRP channels and Ca²⁺ signaling. *Cell Calcium*, **40**, 261-75.

Nadler MJ, Hermosura MC, Inabe K, et al (2001). LTRPC7 is a Mg.ATP-regulated divalent cation channel required for cell viability. *Nature*, **411**, 590-5.

Paravicini TM, Chubanov V, and Gudermann T. (2012). TRPM7: a unique channel involved in magnesium homeostasis. *Int J Biochem Cell Biol*, **44**, 1381-4.

Pham DL, Scheble V, Bareiss P, et al (2013). SOX2 expression and prognostic significance in ovarian carcinoma. *Int J Gynecol Pathol*, **32**, 358-67.

Pitakkarnkul S, Tangjitgamol S, Srijaipracharoen, et al (2013). Treatment outcomes of paclitaxel for refractory or recurrent epithelial ovarian cancer patients in Thailand. *Asian Pac J Cancer Prev*, **14**, 2421-7.

Prevarskaya N, Zhang L, and Barritt G. (2007). TRP channels in cancer. *Biochim Biophys Acta*, **1772**, 937-46.

Qiu JJ, Guo JJ, Lv TJ, et al (2013). Prognostic value of centromere protein-A expression in patients with epithelial ovarian cancer. *Tumour Biol*, **34**, 2971-5.

Ramsey IS, Delling M, and Clapham DE. (2006). An introduction to TRP channels. *Annu Rev Physiol*, **68**, 619-47.

Rybarczyk P, Gautier M, Hague F, et al (2012). Transient receptor potential melastatin-related 7 channel is overexpressed in human pancreatic ductal adenocarcinomas and regulates human pancreatic cancer cell migration. *Int J Cancer*, **131**, E851-61.

Siegel R, Naishadham D, and Jemal A. (2013). Cancer statistics, 2013. *CA Cancer J Clin*, **63**, 11-30.

Thigpen T, duBois A, McAlpine J, et al (2011). First-line therapy in ovarian cancer trials. *Int J Gynecol Cancer*, **21**, 756-62.

Vaughan S, Coward JI, Bast RC, et al (2011). Rethinking ovarian cancer: recommendations for improving outcomes. *Nat Rev Cancer*, **11**, 719-25.

Wang B, Liu SZ, Zheng RS, et al (2014). Time trends of ovarian cancer incidence in china. *Asian Pac J Cancer Prev*, **15**, 191-3.

Yee NS, Chan AS, Yee JD, and Yee RK. (2012a). TRPM7 and TRPM8 ion channels in pancreatic adenocarcinoma: potential roles as cancer biomarkers and targets. *Scientifica*, **2012**, 415158.

Yee NS, Zhou W, Lee M, and Yee RK. (2012b). Targeted silencing of TRPM7 ion channel induces replicative senescence and produces enhanced cytotoxicity with gemcitabine in pancreatic adenocarcinoma. *Cancer Lett*, **318**, 99-105.