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ORIGINAL ARTICLE

High expression of mitochondrial intermembrane chaperone TIMM9 represents a negative prognostic marker in gastric cancer

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Background/Purpose: Gastric cancer (GC) is one of the most common malignant cancers worldwide. However, little is known about the molecular process underlying this disease and its progression. This study investigated correlations between the expression of a mitochondrial inner membrane protein translocase of inner mitochondrial membrane 9 homolog (TIMM9) and various clinicopathologic parameters as well as patients' survival.
Methods: Gastric tissue samples were obtained from 140 patients with GC and expression levels of TIMM9 were analyzed through immunohistochemistry. Paired *t* tests were used to analyze the differences in the expression levels of TIMM9 in both tumor and nontumor tissues for each patient. Two-tailed χ^2 tests were performed to determine whether the differences in

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TIMM9 expression and clinicopathologic parameters were significant. Time-to-event endpoints for clinicopathologic parameters were plotted using the Kaplan–Meier method, and statistical significance was determined using univariate log-rank tests. Cox proportional hazard model was used for multivariate analysis to determine the independence of prognostic effects of TIMM9 expression.

Results: A borderline association was found between overexpression of TIMM9 and vascular invasion ($p = 0.0887$). Patients with high expression levels of TIMM9 achieved a significantly lower disease-free survival rate compared with those with low expression levels ($p = 0.005$). Multivariate Cox regression analysis showed that overexpression of TIMM9 was an independent prognostic marker for GC ($p = 0.011$).

Conclusion: Overexpression of TIMM9 can be used as a marker to predict the outcome of patients with GC.

Introduction

Gastric cancer (GC) ranks as the second most frequent cause of cancer-related mortality.[1] In Taiwan, GC results in more than 2000 deaths annually (http://www.mohw.gov.tw/CHT/DOS/Statistic.aspx?f_list_no=312&fod_list_no=6201). The outcome of this common malignancy remains unsatisfactory because of poor understanding of the pathogenesis of GC and the lack of specific target gene therapy.[2,3] In recent decades, several studies have suggested that genetic alterations may play a role in the development and progression of GC.[4] Studies in molecular pathology, both ours and others, may help clarify the pathogenesis of GC and may reveal useful prognostic molecular markers.[5–10]

An elaborate mitochondrial translocation system is necessary to transport protein precursors to their correct intramitochondrial destinations.[11,12] Cancer cells amplify their capacity for mitochondrial oxidative metabolism and "steal" high-energy mitochondrial fuels from adjacent stromal cells.[13,14] In direct support of this hypothesis, genetic induction of mitochondrial dysfunction in cancer-associated fibroblasts dramatically promotes both local tumor growth and distant cancer cell metastasis.[15,16] Translocases of inner mitochondrial membrane (TIMM) family consists of a group of evolutionarily conserved proteins that participate in the import and insertion of multipass transmembrane proteins into the mitochondrial inner membrane.[17] Proteomic analysis showed an increase of TIMM17A protein levels in breast cancer cells, and the increase was validated by immunoblotting and immunohistochemistry. Ductal carcinoma *in situ* and invasive ductal carcinoma of the breast displayed strong staining, whereas the adjacent normal epithelia and stromal cells displayed negative staining.[18] Similarly, Sotgia et al[19] demonstrated that 15 markers of mitochondrial biogenesis, including TIMM9 and TIMM17A, selectively labeled epithelial breast cancer cells and were largely absent from adjacent tumor stromal cells.

The clinical importance of TIMM family has gained increasing attention in recent years, with reports suggesting that TIMM may contribute to the clinical outcome. Studies by both Xu et al[18] and Salhab et al[20] have reported that TIMM17A expression was significantly associated with unfavorable pathological parameters, including tumor grade, nodal positivity, and stage, as well as with adverse clinical outcomes such as overall and disease-free survival. Thus, TIMM17A is a promising diagnostic and prognostic marker for breast cancer patients.

To date, the prognostic significance of TIMM9 expression level in human GC has not been established. This study investigated the correlations between TIMM9 expression and clinicopathologic parameters, and evaluated the significance of TIMM9 in predicting the prognosis for patients with GC.

Materials and methods

Ethics statements

The Institutional Review Board at Wan Fang Hospital, Taipei, Taiwan, approved the tissue acquisition protocol for the immunohistochemical, immunoblotting, and quantitative real-time polymerase chain reaction (PCR) study (Approval Number: 99049). Written informed consent was obtained from each participant before tissue acquisition.

Participants and specimens

The patient cohort comprised 140 consecutive GC cases from 1998 through 2011 documenting pathologic and clinical factors and clinical outcome. All the cases in this study received radical total or subtotal gastrectomy with D2 or D3 lymph node dissection. None of our study patients had received preoperative chemotherapy and/or radiotherapy. The nontumor portion was obtained from grossly normal gastric mucosa, separate from the tumor, in resected gastric specimen. Clinicopathologic parameters of GCs were determined according to the American Joint Committee on Cancer (AJCC) classification. The follow-up duration for disease-free survival was defined as the period between the operation date and the day of relapse, according to the patient's chart. For each patient, we