Expression profiles of mTOR pathway proteins in porocarcinoma: A provisional immunohistochemical study

MITSUAKI ISHIDA and HIDETOSHI OKABE

Department of Clinical Laboratory Medicine and Division of Diagnostic Pathology, Shiga University of Medical Science, Otsu, Shiga 520-2192, Japan

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Abstract. Porocarcinoma is a rare skin appendage carcinoma, with a poor prognosis. At present, the recommended treatment of localized porocarcinoma is wide surgical resection. Although anthracyclin-based chemotherapy or combination of 5-fluorouracil (5-FU), taxanes and cisplatin are considered to be the first-line treatment for metastatic or locally-advanced porocarcinoma, this type of tumor is recognized as relatively chemoresistant, and no standard systemic treatment has been established yet. Mammalian target of rapamycin (mTOR) is an important protein involved in carcinogenesis. mTOR phosphorylates the eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), and then phosphorylated 4E-BP1 (p4E-BP1) triggers cell cycle progression, cell proliferation and angiogenesis. Therefore, mTOR is believed to be one of the most promising therapeutic targets in various types of carcinomas. However, the expression profiles of mTOR pathway proteins in porocarcinoma have yet to be elucidated. Therefore, we analyzed the expression of mTOR, 4E-BP1 and p4E-BP1 in five cases of porocarcinoma (four invasive and one *in situ* case) using immunohistochemical methods. mTOR expression was observed in the invasive porocarcinoma cases, but not in the *in situ* case. 4E-BP1 was expressed in all five cases. p4E-BP1 expression was observed in 3/4 invasive porocarcinoma cases, but not in the *in situ* case. This preliminary study clearly demonstrated the overexpression of mTOR and its downstream proteins in most of the included invasive porocarcinoma cases. Therefore, mTOR inhibitors could be considered as potential therapeutic modalities for the treatment of metastatic or locally-advanced porocarcinoma.

Correspondence to: Dr Mitsuaki Ishida, Department of Clinical Laboratory Medicine and Division of Diagnostic Pathology, Shiga University of Medical Science, Tsukinowa-cho, Seta, Otsu, Shiga 520-2192, Japan
E-mail: mitsuaki@belle.shiga-med.ac.jp

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Introduction

Porocarcinoma is a rare skin appendage carcinoma related to the sweat gland duct and is known to be detected in 0.004% of skin biopsy specimens (1). Its prognosis is not favorable due to the fact that it carries a significant risk of local recurrence, as well as lymph node and distant metastases (2,3). At present, the recommended treatment of localized porocarcinoma is wide surgical resection and in the cases where lymph node metastasis is present, radical lymph node dissection is added. Although anthracylin-based chemotherapy or combination of 5-fluorouracil (5-FU), taxanes and cisplatin are considered to be the first-line treatment for metastatic or locally-advanced porocarcinoma, this type of tumor is recognized as relatively chemoresistant, and no standard systemic treatment exists (4).

Mammalian target of rapamycin (mTOR) is a key protein involved in carcinogenesis and is activated via the phosphatidylinositol-3 kinase (PI3K)/AKT pathway. mTOR phosphorylates the eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), and then the phosphorylated 4E-BP1 (p4E-BP1) triggers cell cycle progression, cell proliferation and angiogenesis (5). Therefore, mTOR is believed to be one of the most promising therapeutic targets in various types of carcinomas. Recently, mTOR inhibitors have been demonstrated to prolong the progression-free survival of patients with pancreatic neuroendocrine tumor (6). Moreover, the expression levels of mTOR pathway proteins have been suggested to be predictive markers to gauge response to treatment with mTOR inhibitors (7). However, the expression profiles of mTOR pathway proteins in porocarcinoma have yet to be elucidated. In this preliminary study, we investigated the expression profiles of mTOR, 4E-BP1 and p4E-BP1 in porocarcinoma cases, and discussed whether or not mTOR inhibitors are suitable candidates for the treatment of porocarcinoma.

Materials and methods

Expression of mTOR, 4E-BP1 and p4E-BP1 was analyzed in five cases of porocarcinoma (four invasive and one *in situ* case), using immunohistochemical methods. Immunohistochemical analyses were carried out using an autostainer (XT system Benchmark; Ventana Medical Systems, Tucson, AZ, USA),

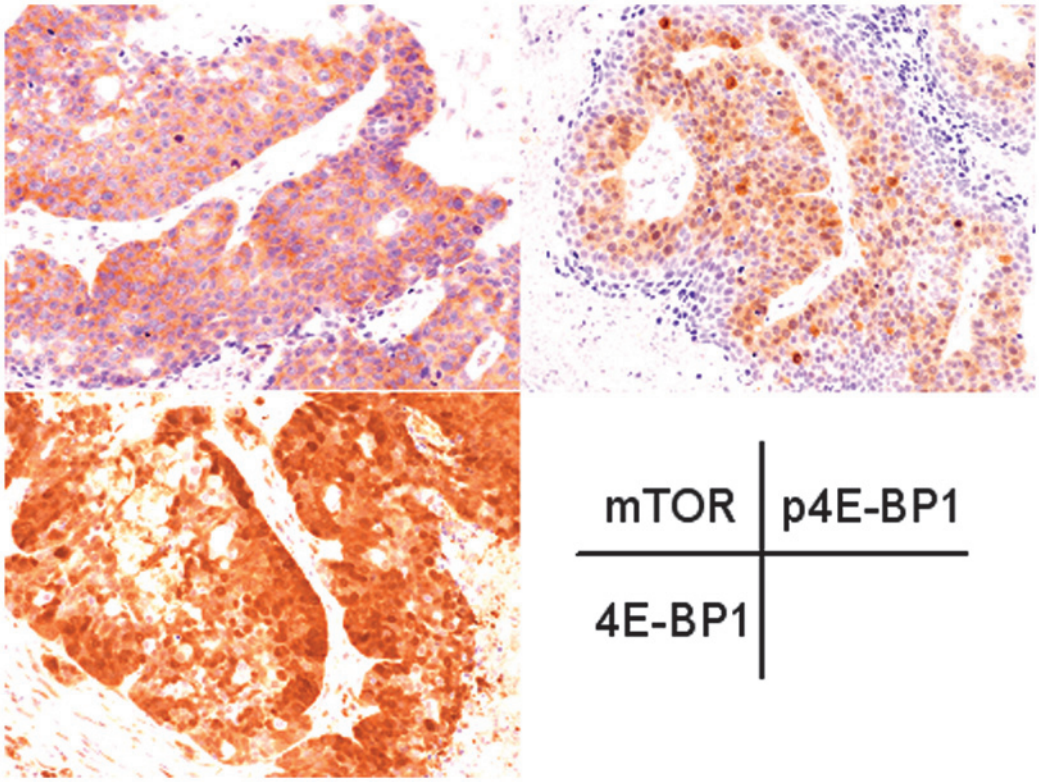

Figure 1. Immunohistochemical staining for mTOR and its downstream proteins in porocarcinoma are shown. mTOR and p4E-BP1 expressed in the cytoplasm of invasive porocarcinoma cells. 4E-BP1 expressed in the cytoplasm of porocarcinoma cells.

according to the manufacturer's instructions. The following primary antibodies were used: a rabbit monoclonal antibody against mTOR (7C10; Cell Signaling Technology, Inc., Danvers, MA, USA), a rabbit monoclonal antibody against 4E-BP1 (53H11; Cell Signaling Technology, Inc.) and a rabbit monoclonal antibody against p4E-BP1 (Thr 37/46) (236B4; Cell Signaling Technology, Inc.).

The immunostaining procedures of these markers were scored using semi-quantitative scoring, as described in a previous study (8).

Results

Table I shows the immunohistochemical staining results of the mTOR pathway proteins. The expression of mTOR was observed in the invasive porocarcinoma cases, but not in the *in situ* porocarcinoma case. 4E-BP1 was expressed in the invasive and *in situ* porocarconima cases. Cytoplasmic p4E-BP1 expression was observed in 3/4 invasive porocarcinoma cases, but not in the *in situ* porocarcinoma case (Fig. 1).

Table I. Expression patterns of mTOR pathway proteins in porocarcinoma cases (n=5).

	mTOR pathway proteins		
Porocarcinoma cases	mTOR	4E-BP1	p4E-BP1
Invasive (n=4)	4/4	4/4	3/4
In situ (n=1)	0/1	1/1	0/1

mTOR, mammalian target of rapamycin; 4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1; p4E-BP1, phosphorylated 4E-BP1.

Discussion

To the best of our knowledge, this is the first study to investigate the expression profiles of the mTOR pathway proteins in porocarcinoma, and the overexpression of mTOR and its downstream proteins in most of the included invasive porocarcinoma cases was clearly demonstrated. Activation of the mTOR pathway has been suggested to be highly involved in the pathogenesis of extramammary Paget's disease (9), as well as in the carcinogenesis of porocarcinoma, especially during the invasive stage.

Moreover, findings of a previous study demonstrated that treatment with mTOR inhibitors led to growth inhibition and cell cycle arrest in the G1 phase of carcinoma cells (10). The present study clearly demonstrated that the mTOR pathway was activated in most of the included invasive porocarcinoma cases. This finding, together with the findings of previous studies, (6,10) demonstrate that mTOR inhibitors are potential therapeutic modalities for the treatment of metastatic or locally-advanced porocarcinoma. Additional clinicopathological studies as well as clinical trials concerning treatment with mTOR inhibitors in patients with porocarcinoma are required to provide more definite evidence.

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