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PAX2 expression is correlated with better survival in tamoxifen-treated breast carcinoma patients

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ABSTRACT

PAX2 (paired box gene 2) is a transcription factor, which is involved in both cell proliferation and carcinogenesis. This study aimed to investigate PAX2 expression in tamoxifen resistant (TAM-R) and tamoxifen sensitive (TAM-S) breast carcinoma patients and analyze its correlation with clinicopathological characteristics and survival. Immunohistochemical analysis was performed to evaluate PAX2 protein expression in 36 TAM-R and 36 TAM-S formalin-fixed paraffin-embedded (FFPE) breast tumor tissues. Data analysis indicated that PAX2 expression was significantly higher in TAM-S group in comparison to TAM-R ($P = 0.014$). Overexpression of PAX2 was significantly correlated with perineural invasion (PNI) ($P = 0.025$). Kaplan–Meier survival analysis showed significant association between high expression of PAX2 and better disease-free survival ($P < 0.001$) and also overall survival ($P = 0.031$). Multivariate cox regression analysis demonstrated that patients with increased expression of PAX2 have a trend toward improved disease free survival (OR = 0.065, 95% CI: 0.009–0.476; P = 0.007) and overall survival (OR = 0.147, 95% CI: 0.020-1.105; P = 0.062). Our data suggested that high expression of PAX2 could be associated with better survival in estrogen receptor positive tamoxifen-treated breast carcinoma patients.

1. Introduction

Breast cancer is an important major health concern in women worldwide (Tanic et al., 2012). Breast carcinoma patients routinely undergo surgery, radiotherapy, and chemotherapy. Hormone therapy is included in the treatment protocol of hormone receptor positive patients. About 70% of breast tumors are estrogen receptor positive (ER^+) (Johansson et al., 2013) and tamoxifen(TAM) is routinely used as anti-estrogen treatment in this group of patients for about 40 years. Furthermore, tamoxifen has been used as a chemo-preventive agent in normal women with a history of inherited breast cancer in their family (Funahashi, 2000). This famous drug, pharmacologically, is a selective estrogen modulator (SERM), which reduces recurrence rate of disease through apoptosis induction (Gelmann, 1996; Mandlekar and Kong, 2001) and blocking ER signaling pathway (Yu and Bender, 2001). Unfortunately, about one-third of tamoxifen-treated patients experience relapse in their treatment period (Musgrove and Sutherland, 2009). For the best management of patients' treatment, it is important to predict clinical outcome prior to disease recurrence and metastasis. Progression of tamoxifen resistance is a very complicated process with includes different genes and signaling pathways e.g. ER signaling pathway, overexpression of oncogenes (e.g. MUC1-C (Mucine-1)(Kharbanda et al., 2013), Mcl-1 (myeloid leukemia cell differentiation)(Thrane et al., 2015), AGR2 (anterior gradient-2) (Hrstka et al., 2010) and Bcl-2 (B-cell lymphoma 2) and repression of tumor suppressor genes such as p*53, retinoblastoma (Rb), and PTEN* (Phosphatase and tensin homolog). Interestingly, overexpression of ErbB2/HER2 as a growth factor receptor takes an important role in progression of TAM resistance in

Abbreviations: PAX2, paired box gene 2; ER^+, estrogen receptor positive; TAM, tamoxifen; TAM-S, tamoxifen sensitive; TAM-R, tamoxifen resistance; DFS, disease free survival; OS, overall survival

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Table 1
Clinicopathological characteristics of breast cancer patients.

Feature	Categories	Number of patients	Percent (%)
Histological grade (MBR [a])			
	Grade I	25	34.7
	Grade II	34	47.2
	Grade III	13	18.1
T stage			
	T1	9	12.5
	T2	44	61.1
	T3	17	23.6
	T4	2	2.8
N stage			
	N0	22	30.5
	N1	21	29.2
	N2	18	25
	N3	11	15.3
Extracapsular nodal extension (ECE)			
	Yes	15	20.8
	No	57	79.2
DCIS histology			
	Comedo type	9	12.5
	Non-Comedo type	63	87.5
Nipple Involvement			
	Yes	13	18.1
	No	59	81.9
Lymphatic invasion			
	Yes	55	76.4
	No	17	23.6
Perineural invasion (PNI)			
	Yes	30	41.7
	No	42	58.3
ER- status			
	Positive	72	100
	Negative	0	0
PR status			
	Positive	47	65.3
	Negative	25	34.7
HER-2 status			
	Positive	19	26.4
	Negative	53	73.6
p53 status			
	Positive	23	31.9
	Negative	49	68.1

[a] MBR: Modified-Bloom-Richardson.

patients (Fernandez-Cuesta et al., 2011; Shou et al., 2004). PAX2 localizes to human chromosome band 10q24.3-q25.1(Izzedine et al., 2003) and is a member of class III of the *PAX* family gene. *PAX* family encode nuclear transcription factors of developmental control genes (Fabian et al., 2015). PAX2 expression is critical during embryonic development of important human body systems including brain and spinal cord (central nervous system), kidney, eye, ear and urogenital tract (Eccles et al., 2002; Porteous et al., 2000). Overexpression of PAX2 has been observed during nephrogenesis, which is required for tubular branching and development(Dressler et al., 1990). Furthermore, PAX2 works as a transcriptional repressor of p53 and activator of Wilms' tumor suppressor gene (WT1) (Dehbi et al., 1996). Overexpression of transcription factors is one of the important primary molecular mechanisms in tumorigenesis; PAX2 also could work as an oncogene (Rabbitts, 1994), which is involved in various malignancies including lung cancer (Ren et al., 2015), renal tumors (Ozcan et al., 2012), ovarian cancer (Wang et al., 2015), melanoma (Lee et al., 2011), etc. The role of PAX2 in embryo development and oncogenesis suggest that it works as a regulatory factor in cell growth. In human body PAX2 expression is normally turned off after terminal differentiation. Interestingly, PAX2 also works as a negative regulator of *ERBB2/HER-2* gene, which keeps *ErbB2* gene off. It has been demonstrated previously that silencing of PAX2 in breast cancer cell line leading to elevated mRNA and protein expression levels of ErbB2 in the presence of both estrogen and tamoxifen treatment (Hurtado et al., 2008). In the present study, we explore PAX2 protein expression and its clinicopathological significance in tamoxifen treated ER^+ breast carcinoma patients.

2. Materials and methods

2.1. Tissue specimens

The medical records of breast cancer patients of Iran National Tumor Bank were reviewed. Breast cancer patients who were undergone breast surgeries from 2005 to 2014 were considered.

Women with ER-positive invasive ductal carcinoma of the breast, who had undergone surgery, radiotherapy, chemotherapy and finally received adjuvant tamoxifen as anti-hormone treatment for 6 months to 5 years or more, were recruited. ER-negative breast tumors or patients with prior neoadjuvant therapy were excluded from this study. A retrospective case–control study was designed and 72 Formalin-Fixed Paraffin-Embedded (FFPE) breast tumor blocks and the corresponding clinicopathological data were retrieved from Iran National Tumor Bank, which is founded by Cancer Institute of Tehran University of Medical Sciences, for Cancer Research. Each patient had signed informed consent for medical record review and sample donation. Regarding to disease recurrence, after 6 months of tamoxifen treatment period all participants were divided into 2 groups tamoxifen resistance (TAM-R) and tamoxifen sensitive (TAM-S). Disease recurrence was defined as local or regional recurrence or distant metastasis and breast cancer-related death. All patients have been followed up for disease free survival and overall survival. TAM-S patients had received standard adjuvant tamoxifen treatment for 5 years or more without any signs of disease recurrence, while all TAM-R patients experienced cancer recurrence (local or regional recurrence, distant metastasis (bone, liver, lung metastasis) or death when receiving tamoxifen treatment for at least 6 months. Each block underwent Hematoxylin and Eosin (H&E) staining and immunohistochemical analysis and histological subtyping, grading, and assessment of ER, PR, HER-2, and p53 status were

Table 2
PAX2 protein expression in TAM-R and TAM-S invasive ductal carcinoma of the breast.

			PAX2 expression					
	Patients	n	− n (%)	+ n (%)	++ n (%)	+++ n (%)	PR[a], %	*p*-value
PAX2	**TAM-R**	**36**	19 (52.8)	0 (0)	16 (44.4)	1 (2.8)	17(0.47)	0.014
	TAM-S	**36**	14(38.9)	0 (0)	6(16.7)	16(44.4)	22(0.61)	

− negative; + weak; ++ moderate; +++ strong staining.
[a] PR, positive rate.