Human Pathology (2013) **44**, 2028–2037

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Human PATHOLOGY

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Original contribution

# Fibronectin expression in carcinoma cells correlates with tumor aggressiveness and poor clinical outcome in patients with invasive breast cancer☆,☆☆

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Received 27 November 2012; revised 20 March 2013; accepted 22 March 2013

**Keywords:**
Fibronectin;
Breast neoplasms;
Immunohistochemistry;
Prognosis

**Summary** Fibronectin (FN), a large heterodimeric glycoprotein, can be found in soluble form in plasma or in insoluble form as an extracellular matrix protein. Cellular FN is produced by various types of benign and malignant epithelial and mesenchymal cells and is widely distributed in malignant tumors. We evaluated FN expression in cancer cells (epithelial FN; E-FN) and intratumor stroma (stromal FN, S-FN) of 1596 invasive breast cancer samples using immunohistochemistry on tissue microarrays. Correlations of FN expression with clinicopathologic factors and patient survival were investigated. Among 1512 informative cases, E-FN expression was observed in 355 (23.5%) cases, and S-FN expression showed no/weak staining in 362 (23.9%), moderate staining in 744 (49.2%), and strong staining in 406 (26.9%) cases. E-FN expression was correlated with advanced pT ($P < .001$) and pN ($P < .001$), histologic type ($P = .006$), high histologic grade ($P < .001$), lymphovascular invasion ($P < .001$), hormone receptor negativity ($P < .001$), and human epidermal growth factor receptor-2 (HER2) positivity ($P < .001$). Strong S-FN expression showed an association with advanced pN ($P = .002$), histologic type ($P < .001$), high histologic grade ($P < .001$), lymphovascular invasion ($P < .001$), and HER2 positivity ($P < .001$). Patients with E-FN expression showed worse overall survival ($P < .001$) and disease-free survival ($P < .001$) than did those with negative expression of FN. E-FN expression was an independent prognostic factor, especially in the hormone receptor–positive group. Expression of S-FN did not have a significant effect on patient survival. In conclusion, E-FN expression could be a promising prognostic marker in patients with invasive breast cancer.


## 1. Introduction

Fibronectin (FN), a large heterodimeric glycoprotein, can be found in a soluble form of plasma or in an insoluble, cellular form as an extracellular matrix protein. The plasma form of FN is produced mainly by hepatocytes, whereas the cellular FN is produced by various benign and malignant

☆ Funding disclosure: This research was supported by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (Grant No. 2012R1A1A3013460).

☆☆ The authors declared no conflict of interest.

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http://dx.doi.org/10.1016/j.humpath.2013.03.006

epithelial and mesenchymal cells and deposited by these cells as filaments into an extracellular matrix [1].

FN plays important roles in cell-matrix and cell-cell adhesion, cell migration, morphogenesis, differentiation, and oncogenic transformation [2]. It is known to activate the PI3K/Akt pathway through binding to its integrin receptor $\alpha_5\beta_1$ in several cancers including breast and lung cancers [3,4]. Han et al [3] reported that FN stimulates proliferation of non–small cell lung carcinoma cells by activation of Akt, an upstream positive modulator of the molecular target of rapamycin (mTOR), and phosphorylation of p70S6K and 4E-BP1, 2 downstream targets of mTOR. Rapamycin, an inhibitor of mTOR, blocked FN-induced phosphorylation of p70S6K and 4E-BP1. They also reported on reduction of the protein level of PTEN by FN. PTEN is an antagonist for protein tyrosine kinases such as PI3K/Akt; thus, loss of PTEN results in phosphorylation of Akt with subsequent stimulation of cell proliferation [5]. Several studies have reported a positive correlation between FN expression and aggressive behavior of lung cancer [6-8].

Epithelial-mesenchymal transition (EMT) of epithelial tumor is defined as having a transient mesenchymal appearance (loss of epithelial characteristics and acquisition of a mesenchymal phenotype). EMT is associated with progression and invasion of cancer cells into the surrounding microenvironment and a chemoresistant phenotype [9]. FN, which is up-regulated during the EMT process in epithelial cancer cells, has been used as a mesenchymal marker for detection of EMT phenotype of cancer [9,10].

Immunohistochemical expression of FN has been reported in several types of cancer including breast cancer, lung cancer, thyroid cancer, and esophageal cancer [2,10-12]. Expression of FN in cases of breast cancer was usually estimated in the tumor stroma and showed inconsistent results for clinical significance of stromal FN (S-FN) expression [2,13-15]. Epithelial FN (E-FN) expression in breast cancer cells has also been studied; however, its clinical significance has not been reported [2].

In an attempt to clarify the prognostic significance of FN expression in invasive breast cancer, using immunohistochemistry (IHC) on tissue microarrays (TMAs), we assessed the expression of E-FN and S-FN in a large cohort of patients with invasive breast cancer. We analyzed the correlation of FN expression with clinicopathologic parameters and patient survival.

## 2. Materials and methods

### 2.1. Case selection and construction of TMA blocks

For this study, we collected 1596 cases of primary invasive breast cancer that had undergone surgical resection at Yeungnam University Hospital, Daegu, South Korea, between January 1995 and December 2007. All tissues were fixed in 10% buffered formalin and embedded in paraffin. We reviewed the slides of all cases and selected a representative tumor block for each case for construction of TMAs. A pair of 2-mm-diameter tissue cores were retrieved from each tumor block and transferred to the recipient block. Thirteen TMA blocks were created from 594 cases, as described previously [16]. The remaining 43 TMA blocks were constructed with 1002 cases (between 2004 and 2007) using a Quick-Ray Manual Tissue Microarrayer (Unitma, Seoul, Korea) and Quick-Ray recipient blocks of 2-mm cores (Unitma).

The age of patients at the time of initial diagnosis, pathologic tumor stage (pT) [17], pathologic lymph node stage (pN) [17], histologic grade [18], presence or absence of lymphovascular invasion, estrogen receptor (ER) and progesterone receptor (PR) status, surgery type, adjuvant treatment, and follow-up information, including patient outcome, were collected from the pathology reports and patients' medical records. For cases that did not have hormone receptor status based on IHC in their pathology reports, we used IHC results for ER and PR obtained by our previous study [19]. Before publication of the current guidelines in 2010 [20], the threshold for ER and PR positivity was 10% or more of tumor cells positive at any intensity. Overall survival (OS) was defined as the time interval between the date of surgical resection and the date of death or last follow-up. Disease-free survival (DFS) was expressed as the number of months from surgical resection to the date of documented relapse, including locoregional recurrence and distant metastasis. This study was approved by the institutional review board of Yeungnam University Hospital (YUH-12-0344-O20).

### 2.2. Immunohistochemistry

IHC was performed on the automated Benchmark platform (Ventana Medical Systems, Tucson, AZ) according to the manufacturer's recommendations. Immunostaining of TMA sections measuring 4 $\mu$m in thickness was performed for FN (clone F14, prediluted, rabbit monoclonal; Biogenex, San Ramon, CA) and human epidermal growth factor receptor-2 (HER2; CONFIRM anti-HER2/neu (4B5) rabbit monoclonal; Ventana Medical Systems) using an UltraView universal DAB detection kit (Ventana Medical Systems). We performed silver-enhanced in situ hybridization using an INFORM HER2 DNA probe (Ventana Medical Systems) for equivocal cases in HER2 IHC, as described previously [16].

Results of FN expression were estimated separately in cancer cells and in the intratumor stroma. For E-FN expression, we considered both staining intensity and the proportion of stained tumor cells [10]. Staining intensity was classified as follows: weak (1), moderate (2), or strong (3). Positive cells were quantified as a percentage of the total number of tumor cells and assigned to 1 of the following 5 categories: less than 5% (0), 5% to 25% (1), 26% to 50% (2), 51% to 75% (3), or more than 75% (4). The percentage of tumor cell positivity and staining intensity was multiplied to generate an immunoreactivity score (IS) for each case. As a result, IS values ranged from 0 to 12 (Fig. 1). Using the