NEOPLASIA
www.neoplasia.com

Volume 16 Number 10 October 2014 pp. 801–813 801

FLI1 Expression is Correlated with Breast Cancer Cellular Growth, Migration, and Invasion and Altered Gene Expression

Melissa N. Scheiber*, Patricia M. Watson†, Tihana Rumboldt‡,1, Connor Stanley§, Robert C. Wilson¶, Victoria J. Findlay#, Paul E. Anderson§ and Dennis K. Watson *

* Department of Pathology and Laboratory Medicine, The James E. Clyburn Research Center, Medical University of South Carolina, 68 President Street, Charleston, SC 29425; † Department of Medicine, Division of Hematology/Oncology, The James E. Clyburn Research Center, Medical University of South Carolina, 68 President Street, Charleston, SC 29425; ‡ Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Children's Hospital, 171 Ashley Avenue, Charleston, SC 29425; § Department of Computer Science, College of Charleston, Charleston, SC 29424; ¶ Department of Pathology and Laboratory Medicine, Medical University of South Carolina, The James E. Clyburn Research Center, Medical University of South Carolina, 68 President Street, Charleston, SC 29425; # Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Walton Research Building, 39 Sabin Street, Charleston, SC 29425

Abstract

ETS factors have been shown to be dysregulated in breast cancer. ETS factors control the expression of genes involved in many biological processes, such as cellular proliferation, differentiation, and apoptosis. FLI1 is an ETS protein aberrantly expressed in retrovirus-induced hematological tumors, but limited attention has been directed towards elucidating the role of FLI1 in epithelial-derived cancers. Using data mining, we show that loss of FLI1 expression is associated with shorter survival and more aggressive phenotypes of breast cancer. Gain and loss of function cellular studies indicate the inhibitory effect of FLI1 expression on cellular growth, migration, and invasion. Using Fli1 mutant mice and both a transgenic murine breast cancer model and an orthotopic injection of syngeneic tumor cells indicates that reduced Fli1 contributes to accelerated tumor growth. Global expression analysis and RNA-Seq data from an invasive human breast cancer cell line with over expression of either FLI1 and another ETS gene, PDEF, shows changes in several cellular pathways associated with cancer, such as the cytokine-cytokine receptor interaction and PI3K-Akt signaling pathways. This study demonstrates a novel role for FLI1 in epithelial cells. In addition, these results reveal that FLI1 down-regulation in breast cancer may promote tumor progression.

Neoplasia (2014) 16, 801–813

Abbreviations: Ad-FLI1, Ad-GFP-FLI1; EMT, Epithelial-mesenchymal transition; ER, Estrogen receptor; FLI1, Friend leukemia virus integration 1; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; GEO, Gene Expression Omnibus; GOBO, Gene expression-based Outcome for Breast cancer Online; IDC, Invasive ductal carcinoma; IHC, Immunohistochemistry; ILC, Invasive lobular carcinoma; N, Normal Breast Tissue; PDEF, Prostate-derived ETS factor; PyVT, FVB/N-Tg(MMTV-PyVT)634Mul/J; Rb, Retinoblastoma; T, Tumor; uPA, Urokinase plasminogen activator

Address all Correspondence to: Dennis K. Watson, PhD, Department of Pathology and Laboratory Medicine, The James E. Clyburn Research Center, Medical University of South Carolina, 68 President Street, Charleston, SC 29425. E-mail: watsondk@musc.edu
[1]Current address: Trg Pignaton 5, 52210 Rovinj, Croatia.

Received 9 July 2014; Accepted 15 August 2014

1476-5586/14
http://dx.doi.org/10.1016/j.neo.2014.08.007

Introduction

Breast cancer is the second most common cancer-related death among women in the United States. In 2014, approximately 232,670 women will be diagnosed with and 40,000 will die from invasive breast cancer [1]. Most breast cancer-related deaths are due to metastatic progression, as cells migrate from the primary tumor, invade, and re-establish at distant sites [2]. With conventional chemotherapies and radiation having minimal effect, metastatic breast cancer is generally incurable [3]. This failure is, in part, due to the fact that breast cancer is not a single disease, but a heterogeneous disease associated with variations in gene expression that misdirect the cancer cell to invade and migrate [3,4]. This transcriptional activation or repression of cancer-associated genes is not clearly understood; however, many ETS family members function as oncogenes or tumor suppressors.

ETS transcription factors are highly conserved proteins with a unique winged helix-turn-helix DNA-binding domain. These proteins recognize a core 5′-GGA(A/T)-3′ sequence present in downstream target genes. ETS factors activate or repress genes involved in various biological processes, including cellular proliferation, differentiation, transformation, and apoptosis [5].

Several ETS factors are dysregulated in breast cancer: ETS1 and ETS2 are both up-regulated in breast cancer [6,7]. PEA3 levels are positively associated with HER2/*neu* overexpressing human breast tumors [8]. The related PEA3 family gene, ERM is positively correlated with EGFR positive breast cancer and with poor overall survival [9]. ESE1 mRNA is overexpressed in DCIS, an early stage of human breast cancer [10]. In contrast, prostate-derived ETS factor (PDEF) is reduced in human invasive breast cancer tissue and absent in invasive breast cancer cell lines [11–13].

Friend leukemia virus integration 1 (FLI1) is a member of the ETS family, initially identified as a proto-oncogene as it is aberrantly-expressed in retrovirus-induced hematological tumors in mice [14]. In humans, FLI1 is rearranged in Ewing's sarcoma and related primitive neuroectodermal tumors characterized by a *t*(11;22)(q24;q12) translocation [15]. FLI1 is essential for embryonic development as its loss results in embryonic lethality due to the absence of megakaryocytes and aberrant vasculogenesis [16–18]. In normal adult tissues, FLI1 is expressed in hematopoietic cells and tissues, endothelial cells and fibroblasts[16,17,19] and along with GATA-1, has a critical role in the regulation of megakaryocyte differentiation [20]. In endothelial cells, FLI1 is important in vascular integrity regulates several vascular homeostasis genes such as VE-cadherin, PECAM1, PDGFB, and SIP_1 receptor [21]. In fibroblasts, the loss of FLI1 results in an increase of collagen type I and a decrease in MMP1, thus contributing to the development of fibrosis [22,23]. FLI1 regulates numerous biological processes in a number of different tissue types; however, limited attention has been directed towards elucidating the potential role of FLI1 in epithelial-derived cancers, including breast cancer.

In this study, we demonstrate that FLI1 is expressed in normal, and decreased in human and mouse mammary tumor tissue and human breast cell lines. Modulation of expression of FLI1 in breast cell lines and animal tumor models alters cell growth, migration, and invasion. Gene expression profiling using RNA-Seq identifies several cellular pathways modified by FLI1 over expression. The results of these experiments provide evidence for a novel and significant role for FLI1 in breast cancer.

Materials and Methods

Cell Culture

Human breast epithelial cell lines were maintained at 37°C with 5% CO_2 in medium supplemented with 10% fetal bovine serum and 100 units penicillin/streptomycin. All cell lines were grown as recommended by their source. MCF-10A and MCF-12A were grown in DMEM/F12 with 5% horse serum, 100 U penicillin/streptomycin, EGF (20 ng/ml), insulin (10 μg/ml), cholera toxin (100 ng/ml), and hydrocortisone (0.5 μg/ml). The breast cancer cell lines CAMA-1, HBL-100, BT-474, and MDA-MB-175VII were a kind gift of R. Neve (Cancer Research Institute, University of California, San Francisco, CA; Lawrence Berkeley National Laboratory, Berkeley, CA), the MCF-10A cell line was a kind gift from B. Toole (Medical University of South Carolina, Charleston, SC), and the MCF-12A cell line was a kind gift from A. Gutierrez-Hartmann (University of Colorado Health Sciences Center, Denver, CO). All other lines were obtained from ATCC. During the course of the experiments described herein, all of the breast cell lines have maintained consistent morphology, doubling times, and tested negative for mycoplasma. MCF-10A and MDA-MB-231 were authenticated in July, 2011 at the Genetics Resource Core Facility at The Johns Hopkins University by using the Power Plex 1.2 system described (http://faf.grcf.jhmi.edu/str.html).

Data Mining

To determine the FLI1 mRNA expression levels in human breast tumors, FLI1 expression values were extracted from the public Gene Expression Omnibus data repository (GEO, http://www.ncbi.nlm.nih.gov/geoprofiles/) [24]. A microarray dataset of 60 micro-dissected estrogen receptor (ER) positive primary breast tumors was selected based on the large sample size [25]. The resulting profile displays the expression level of FLI1 across all samples within DataSet Record GDS807. FLI1 expression values in human breast tumors were also extracted from public datasets from Oncomine 4.4 Research Edition (Compendia Bioscience, Ann Arbor, MI). To find the resulting dataset the following filters were used: Gene: FLI1; Analysis Type: Cancer vs. Normal; Cancer Type: Breast. The data sets were ordered by under-expression: p-values and the datasets selected for analysis and visualization were the top two with the most significant *P* values (Zhao Breast and Richardson Breast 2) [26,27]. Expression levels of FLI1 in 1881 breast cancer samples, broken down into subtypes, and 51 breast cancer cell lines was obtained from the online tool GOBO, Gene expression-based Outcome for Breast cancer Online (http://co.bmc.lu.se/gobo) [28]. In addition, GOBO was used to assess relapse free survival and overall survival based on expression level of FLI1 in these tumors as well as co-expressed gene pathways [28].

Immunohistochemistry

Tissue microarray slides were obtained through Imgenex (San Diego, CA) from SuperBioChips in Korea (Imgenex is their US distributor) (http://www.tissue-array.com/ver3/index.php) and were collected under discarded tissue protocols (tissue in excess of that needed for diagnosis). Additional human breast cancer samples were obtained from the Pathology Department at the Medical University of South Carolina (MUSC, Charleston, SC). These tissues were obtained from consented patients who agreed to have material not needed for pathological diagnosis be available for research purposes. Specimens were provided under an Exempt IV research MUSC IRB for human research (HR 19968, DKW).

All of the specimens were formalin-fixed and paraffin-embedded. Antigen retrieval and immunohistochemical staining were performed as previously described; however, NovaRed was used for the substrate [12]. Anti-FLI1 antibodies, previously prepared [29], were used at 1:800 dilution. All of the sections were examined independently by a