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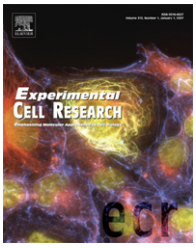

Research Article

S100A7 (psoriasin) influences immune response genes in human breast cancer

Soma Mandal[a,b], *Linda Curtis*[a], *Molly Pind*[a,b], *Leigh C. Murphy*[b,c], *Peter H. Watson*[a,d,*]

[a]*Department of Pathology, University of Manitoba, Winnipeg, Manitoba, Canada*
[b]*Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, Manitoba, Canada*
[c]*Department of Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, Canada*
[d]*Department of Pathology, BC Cancer Agency, Victoria, BC, Canada*

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ABSTRACT

S100A7 (psoriasin) is highly expressed in preinvasive breast carcinomas and in a subset of poor prognosis invasive tumors. To determine the influence of S100A7 expression on ERα negative breast cancer, we profiled mRNA gene expression by Microarray and SAGE analysis, using the ERα negative MDA-MB-231 cell line model. Statistically significant transcripts of genes with very high differential expression were further validated by QPCR in both MDA-MB-231 and MDA-MB-468 cell lines expressing exogenous and endogenous S100A7. S100A7 expression correlated with increases in genes associated with MHC class II receptor activity, antigen processing and antigen presentation, and immune cell activation. The transcription factors (TFs) prediction tool CARRIE confirmed an association between TFs reported to be upregulated by S100A7 (NF-κB, AP-1, and HIF1) and the regulation of many genes in this dataset. The relationship between S100A7 up-regulation and the MHC class II and HLA-class II molecule coding gene CD74 was examined further in a cohort of ERα negative breast tumors by tissue microarray (TMA) and immunohistochemistry (IHC), confirming a significant association *in vivo* ($p=0.042$, $n=149$). These results are consistent with a role for S100A7 in modulating the immune response which may be a factor in early breast tumor progression.

Introduction

S100A7, also known as psoriasin, belongs to the S100 family of EF hand calcium binding proteins. An 11.4 kDa secreted protein, it was originally identified in abnormal keratinocytes of skin affected by psoriasis. Increased S100A7 expression is triggered by inflammatory conditions associated with either abnormal keratinocyte differentiation of the skin [1] or squamous cell malignancies of the skin [2], bladder [3], and breast [4]. Certain other cellular factors such as loss of attachment to extracellular matrix (ECM), growth factor deprivation, and confluency also up-regulate S100A7 expression *in vitro* in the non-tumorigenic (MCF10A) and tumorigenic mammary epithelial cells (MDA-MB-468) [5]. The effect of this upregulation of S100A7 and the mechanism by which S100A7 elicits its biological actions is beginning to be understood. An interaction between S100A7 and the multifunctional intracellular signaling protein Jab1/CSN5 (c-jun

* *Corresponding author*. Deeley Research Centre, BC Cancer Agency, Vancouver Island Center, 2410 Lee Avenue, Victoria, BC, Canada V8R 6V5. Fax: +1 250 519 2009.
E-mail address: pwatson@bccancer.bc.ca (P.H. Watson).

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activation domain binding protein 1) [6] correlates with increased cell survival and invasiveness *in vitro*. These effects may be mediated in part through altered Jab1 activity and increased AP-1, NF-κB and HIF1α driven gene expression [7]. These studies give important, but partial insights to S100A7 mediated effects in breast cancer progression. However, we have previously observed that the tumor promoting effects of S100A7 are more prominent *in vivo* and Jab1 has pleiotropic effects that might influence other cellular pathways [6].

Central to the interest in the role of S100A7 in breast cancer progression is its pattern of overexpression. S100A7 is differentially expressed between stages of breast cancer and it is among one of the most highly expressed genes in preinvasive high-grade DCIS [5,8,9]. It is low to undetectable in normal breast and benign lesions and is often down-regulated in invasive carcinoma [8]. Persistent expression can occur in a subset of invasive tumors where it inversely correlates with time of recurrence and survival [4]. A role of S100A7 in promoting progression has been linked to its ability to enhance pro-survival cellular pathways [9–11] and promote angiogenesis [12]. However, a global perspective is warranted to examine other effects of S100A7 overexpression which might contribute to breast cancer progression. In this study, we have used global gene techniques such as microarray (MA) and serial analysis of gene expression (SAGE) to profile the influence of S100A7 on gene expression in a breast cancer cell line model. The global gene expression data generated from this study has enabled us to further explore the possible role of S100A7 in the context of the ERα negative breast cancer phenotype, most commonly associated with S100A7 expression.

Materials and methods

Materials

The human breast cancer cell lines, MDA-MB-231 (wt 231) and the MDA-MB-468 were obtained from the American Type Culture Collection (Rockville, MD, USA). The MDA-MB-231 cells, engineered to constitutively overexpress S100A7 has been previously described [6]. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were obtained from Invitrogen (CA). Triazol reagent for total RNA extraction was obtained from Invitrogen. For cDNA preparations and PCR, the reverse transcription kit, Platinum Taq Polymerase and primers were obtained from Invitrogen. MA (Affymetrix GeneChip Microarray) service was provided by ParadigmArrayLabs (Icoria, NC) and the I-SAGE kit was obtained from Invitrogen. Purification and sequencing of PCR products was done using the MEGA-Base automated sequencer at the Institute of Marine BioSciences, National Research Council (Halifax, NS, Canada). SYBR green was from Sigma Chemical Co. and fluorescein was from BIORAD.

Tissue culture, MA and SAGE analysis

The ERα negative human breast cancer cell lines MDA-MB-231 (wt-231), MDA-MB-231-HP2 (231-HP2), and MDA-MB-468 were cultured in DMEM supplemented with 5% FBS under standard conditions [13]. We reconfirmed the absence of S100A7 expression in wt-231 cells and the presence of high levels of S100A7 mRNA and protein (by PCR and Western blot, data not shown) in 231-HP2 and MDA-MB-468 cells as reported [5]. Total RNA that was extracted from the cell lines were used for MA, SAGE, and PCR analyses respectively. MA was done using the Human U133A Plus 2.0 gene chip, which gives a complete coverage of the human genome (54,675 probe ID sets) (www.affymetrix.com). SAGE libraries were constructed as described with minor modifications [14]. PCR products from positive clones were purified and sequenced for tag-to-gene extraction by the SAGE software.

Data processing and statistical analysis of SAGE libraries

SAGE tags were extracted from raw sequence data and statistical analysis (p chances) was performed using SAGE2000 (v4.5) software (courtesy Professor Kinzler, John Hopkins University). Tag-to-gene assignment was done by comparing with the reference library (www.ncbi.nlm.nih.gov/SAGE/SAGEtag.cgi) in MS Access. A total of 20,694 tags were obtained from two libraries (wt-231 and 231-HP2); of these, there were 2724 unique tags. Three other statistical tests (IDEG6 software), namely Audic-Claverie, Fisher, and Chi Square tests [15] were used to reconfirm statistically significant differences in gene expression. Genes satisfying the significance criterion ($p \leq 0.05$) by all statistical tests (wt-231/231-HP2) were used. Fold changes in SAGE are given as a ratio of interlibrary tag counts, in this case between our two libraries, wt-231/231-HP2 or 231-HP2/WT-231. Tag counts in SAGE were compared to signal intensities of MA for individual transcripts, after matching probe ID sets (MA) to the unique tags (SAGE) as shown in Table 1. Even global gene expression techniques MA and SAGE differ in their sensitivity and hence reflect differences in the transcript levels. Moreover, change in expression of all transcripts was detected at the mRNA level and further validation at the protein level for most of these genes remains to be done.

During analysis, we found that some tags have more than one gene and some genes have more than one tag. Moreover, one transcript can also have more than one probe ID in MA analysis. After eliminating ESTs, hypothetical protein coding transcripts and duplicate tags (SAGE)/multiple probe IDs (MA) by advanced query function of MS Access and by manual inspection, we obtained 59 highly differentially expressed consensus transcripts, a small subset of which were validated by QPCR (Table 1). Finally, we used extensive data sorting of statistically significant transcripts ($p \leq 0.05$) by both techniques, which yielded an overall number of 321 consensus/non-consensus transcripts, which we used for bioinformatics analyses.

Quantitative Real Time PCR (QPCR)

The expression of a small subset of highly significant differentially expressed consensus transcripts by MA and SAGE were validated by Real Time QPCR. PCR amplification was performed using the iQ Multi Color Real Time PCR Detection System (Bio-Rad Labs, CA). The SYBR green