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Research Article

S14 protein in breast cancer cells: Direct evidence of regulation by SREBP-1c, superinduction with progestin, and effects on cell growth

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Abstract

Most breast cancers exhibit brisk lipogenesis, and require it for growth. S14 is a lipogenesis-related nuclear protein that is overexpressed in most breast cancers. Sterol response element-binding protein-1c (SREBP-1c) is required for induction of lipogenesis-related genes, including S14 and fatty acid synthase (FAS), in hepatocytes, and correlation of SREBP-1c and FAS expression suggested that SREBP-1c drives lipogenesis in tumors as well. We directly tested the hypothesis that SREBP-1c drives S14 expression and mediates lipogenic effects of progestin in T47D breast cancer cells. Dominant-negative SREBP-1c inhibited induction of S14 and FAS mRNAs by progestin, while active SREBP-1c induced without hormone and superinduced in its presence. Changes in S14 mRNA were reflected in protein levels. A lag time and lack of progestin response elements indicated that S14 and FAS gene activation by progestin is indirect. Knockdown of S14 reduced, whereas overexpression stimulated, T47D cell growth, while nonlipogenic MCF10a mammary epithelial cells were not growth-inhibited. These data directly demonstrate that SREBP-1c drives S14 gene expression in breast cancer cells, and progestin magnifies that effect via an indirect mechanism. This supports the prediction, based on S14 gene amplification and overexpression in breast tumors, that S14 augments breast cancer cell growth and survival.

Keywords: Spot 14; THRSP; Progesterone; T47D cells; Breast cancer; SREBP1; CHREBP; Fatty acid synthase

Introduction

S14 is a primarily nuclear protein that is abundant in tissues active in long chain fatty acid synthesis, including lactating mammary gland (reviewed in [1]). We previously demonstrated that the S14 gene on chromosome 11q13 may be amplified in breast cancer cells, and that S14 protein is overexpressed in most breast cancers [2]. Concordant overexpression of S14 and acetyl CoA-carboxylase, the rate-determining enzyme of long chain fatty acid synthesis, indicated that S14 is a component of the lipogenic phenotype observed in aggressive breast cancers. Taken together, the observations of gene amplification, frequent S14 protein overexpression, and association with enhanced lipid metabolism suggested that S14 could influence breast cancer growth. This prediction was strongly supported by our recent analysis of S14 expression in 131 breast cancer cases, which demonstrated striking associations of S14 overexpression with high-grade and bulky tumors, and with reduced disease-free survival [3].

The lipogenic tumor phenotype is characterized by high rates of fatty acid synthesis, elevated tumor content of

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lipogenic enzymes such as fatty acid synthase (FAS), and dependence on lipogenesis for tumor cell growth (reviewed in [4]). The latter was shown by Pizer and coworkers using cerulenin, a pharmacological inhibitor of fatty acid synthase that caused apoptosis of breast cancer cells [5], and inhibited the growth of human ovarian tumor cell xenografts in nude mice [6]. Likewise, the antiobesity drug Orlistat, also a FAS inhibitor, caused apoptosis of lipogenic prostate cancer cells in culture and in xenografts in immunodeficient mice [7].

In hepatocytes, S14 and lipogenic enzymes are inducible by insulin, glucose metabolism, and thyroid hormone (reviewed in [1]). The lipogenic effects of insulin are substantially mediated at the gene level by sterol response element-binding protein 1c (SREBP-1c), a transcription factor that resides in the endoplasmic reticulum until insulin activates its translocation to the Golgi, where the active fragment is released by proteolysis, permitting transit to the nucleus to activate gene transcription ([8], reviewed in [9]). It is attractive to hypothesize that, in breast cancer cells, as in the liver, SREBP-1c is the major driver of lipogenic gene expression. To date, this issue has been addressed in studies of breast cancer specimens [10], colon cancer specimens and cells [11], and prostate cancer cells [12–14]. The studies of breast and colon cancer correlated expression of FAS and SREBP-1c, but did not include mechanistic experiments. Studies in prostate cancer cells, however, directly demonstrated dependence of androgen- and growth factor-induced expression of FAS on SREBP-1c. Moreover, processing of the extranuclear SREBP-1c precursor was increased by androgen induction of SREBP cleavage-activating protein (SCAP), the protein responsible for escorting SREBP-1c to the Golgi, where proteolytic activation occurs. In contrast to the enhancement of SREBP-1c processing by androgen in prostate cancer cells, however, Heemers and coworkers saw no increase in nuclear SREBP-1c content in progestin-treated T47D cells demonstrating S14 gene induction [15].

We have now focused on the regulation of S14 mRNA and protein expression by progestin in breast cancer cells, and the role of SREBP-1c in the action of the hormone. Our results provide mechanistic evidence that induction of S14 mRNA and protein by progestin in breast cancer cells requires the action of SREBP-1c. Several lines of evidence, however, suggest that this action of progestin is not mediated directly. Moreover, we provide evidence for a role of S14 in the growth and survival of breast cancer cells.

Materials and methods

Recombinant adenovirus

Adenovirus harboring dominant-negative and constitutively active SREBP-1c mutants was kindly supplied by F. Foufelle (Paris, France [16]). A full-length rat S14 cDNA ([17]; sense orientation, Ad-S14; antisense orientation Ad-S14-AS) or β-galactosidase gene (negative control; Ad-β-gal) was inserted into adenoviral DNA (Clontech) as described [18]. Viruses were propagated in HEK293 cells (ATCC) and titered by immunocytochemical analysis for a capsid protein (Rapid-Titer, Clontech). The multiplicity of infection (MOI) required for quantitative infection was determined by staining Ad-β-gal-infected wells.

Cell culture and infection

T47D cells (ATCC) were grown in RPMI 1640 plus 10 μg/ml insulin, HEK293 cells (ATCC) in RPMI 1640, and MCF10a cells in DMEM/F12 plus 4 mg/ml insulin, 20 μg/ml epidermal growth factor, and 1 mg/ml hydrocortisone. Media contained penicillin, streptomycin, 4 mM glutamine, 25 mM glucose unless noted otherwise, and 10% fetal calf serum unless noted. Charcoal-stripped fetal calf serum (Hyclone) was used in studies involving R5020 or R1881 (10 nM, New England Nuclear): an equal volume of ethanol vehicle was added to control cultures. Cerulenin (Sigma) was used at 10 μg/ml.

Plasmid transfection

T47D or HEK 293 cells were plated at 50% confluence in 75 cm^2 flasks and the next morning were transfected with 8 μg plasmid DNA in 48 μl Fugene (Roche) in 5% charcoal-stripped serum-containing media without antibiotics. In order to ensure uniform transfection efficiency, cells were trypsinized, mixed, and redistributed into 6 well plates 8 h later: 48 h posttransfection, culture medium was removed, and extracts prepared in reporter lysis buffer (Promega, 250 μl/well). Lysates (20 μl) were assessed for luciferase activity using a LMaxII384 luminometer (Molecular Devices), and normalized to protein concentrations (BCA Protein Assay, Pierce).

Transfection of siRNA

Cells were plated at ~70% confluency in 60 mm dishes the day before transfection with 20 μg siRNA in 333 μl diluent supplied by Qiagen, and 120 μl of RNAifect Transfection Reagent (Qiagen). The siRNAs (Dharmacon) targeted the following sequences in S14 mRNA: siRNA#1: 5′-ggaaatgacgggacaagtt-3′; siRNA#2: 5′-cagccgaggtgcacaacat-3′. Scrambled siRNAs were employed as controls. Complexes were incubated at room temperature for 15 min and added drop-wise to cultures. After 24 h, cells were trypsinized and redistributed into 4 wells of a 12 well plate to ensure uniform transfection efficiency, in media containing hormone or vehicle.

Preparation of anti-S14 antibody and Western blot

Monoclonal antibody against human S14 was prepared in the Norris Cotton Cancer Center antibody resource (Dart-