

Growth Inhibition of Retinoic Acid Treated MCF-7 Breast Cancer Cells-Identification of Sox 9 and Other Proteins

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Background and Significance

Despite advances in treatment, breast cancer continues to be the second leading cause of cancer mortality in women. Statistics suggest that while focus on treatment should continue, chemopreventive approaches should also be pursued¹ SRY and SOX9 are involved in both skeletal development and sex determination,² and have been shown to be nuclear proteins.³ Human SOX4 is expressed in the normal breast and in breast cancer cells. Treatment of T-47D breast cancer cells with the synthetic progestin ORG 2058 directly increased SOX4 transcription. This caused a 4-fold increase in SOX4 mRNA levels within 4 h of treatment.⁴ Retinoids can also reduce expression of the inhibitor of apoptosis protein, survivin.⁵ PDCD4 (programmed cell death 4), a tumor suppressor gene presently being evaluated as a target for chemoprevention, was induced about three-fold by the retinoic acid receptor (RAR α)-selective agonist Am580 in T-47D breast cancer cells. RAR-agonists did not induce PDCD4 expression in breast cancer cell lines, which were not growth inhibited by retinoids.⁶ Retinoic acid (RA) inhibits the growth of MCF-7 mammary carcinoma cells, by involvement of the two proteins that mediate transcriptional activation by RA, the nuclear hormone receptor, retinoic acid receptor (RAR), and the cellular retinoic acid-binding protein (CRABP) II. In MCF-7 mammary carcinoma cells, growth inhibition by RA entails an early cell cycle arrest followed by induction of apoptosis. Expression array analyses revealed that RA induces the expression of several genes involved in cell cycle regulation, including the p53-controlled antiproliferative gene, B-cell translocation gene, member 2 (Btg2). Induction of Btg2 by RA was accompanied by a marked decrease in cyclin D1 expression. This demonstrated that the antiproliferative activity of RA in MCF-7 cells is mediated, at least in part, by Btg2.⁷

We have measured histone H2A directly from breast cancer cells with MALDI.⁸ Recently we identified the following proteins from all-Trans retinoic acid (ATRA, Sigma, St. Louis, Mo) treated MCF-7 breast cancer cells: gi|938234 SOX-9 [Homo sapiens] Mass: 6448; gi|110591141 Chain A, Solution Structure Of The First Homeobox Domain Of At- Binding Transcription Factor 1 (Atbf1) Mass: 7974; gi|5454114 tissue factor pathway inhibitor isoform a precursor [Homo sapiens] Mass: 34992; gi|83715968 sarcoma antigen NY-SAR-79 [Homo sapiens] Mass: 124077; gi|119587536 ferredoxin 1 [Homo sapiens] Mass: 28101; and gi|3851261 immunoglobulin M heavy chain [Homo sapiens] Mass: 10193. Our hypothesis is that the identification of additional proteins will allow characterization of the metabolic cascade induced by ATRA and may lead to therapeutic strategies in breast cancer.

Materials and Methods

MCF-7 breast cancer cell lines were obtained from American Type Tissue Culture Collection (ATCC; Rockville, MD, US). Three groups were treated and three groups were used as controls. Cells were incubated with 1 mM ATRA. Proteins were extracted from the cells with organic solvent and high pressure using ProteoSolve and the Barocycler respectively (Pressure BioSciences, West Bridgewater, MA). The protein fraction was trypsinized in less than 45 minutes, reduced from 12 hour digest time. The peptides were studied with LCMS (Hitachi NanoFrontier nLC, Dallas, TX).

Results

The peptide yield from the protein extraction tryptic digest included a significant number of additional proteins not seen previously. Protein identification was performed with Mascot search of the NCBI nr and Swiss prot databases. Table 1. High pressure extraction increased protein yield and helped to identify proteins not previously seen and revealed marked differences between retinoic acid treated and non-treated cells. The identification of additional proteins will allow characterization of the metabolic cascade induced by RA and may lead to new therapeutic strategies in breast cancer. Table 1

Discussion & Conclusion

The combination of high pressure protein extraction with an organic solvent, and LCMS separation of peptides from the tryptic digest produced both a higher yield of proteins as well as new proteins not previously identified. The high pressure extraction materially reduced the time of trypsin digest. The nanoflow LCMS allowed greater peptide separation and identification of a much larger number of proteins. This demonstrated marked differences between retinoic acid treated and non-treated cells. The low toxicity of RA and its marked apoptotic effect on breast cancer cells offers a new therapeutic strategy for the clinical treatment of breast cancer.

References

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Sample 1

Histone 4 (H4) (Swiss prot) Macrophage migration inhibitory factor (MIF) (Swiss prot)
Heat Shock protein HSP 90 (Swiss prot)
 GI 3287489, Hsp89-alpha-delta-N [Homo sapiens]
 GI 31979, histone H2A.2 [Homo sapiens] GI 40254816, heat shock protein 90kDa alpha (cytosolic), class A member 1 isoform 2 [Homo sapiens]
 GI 34039, unnamed protein product [Homo sapiens]
 GI 31645, glyceraldehyde-3-phosphate dehydrogenase [Homo sapiens]

Sample 2

RA Histone 4 (H4)
Heat shock protein 90kDa alpha (cytosolic), class A member 1 isoform 2 [Homo sapiens] (Swiss Prot)
Heat shock protein HSP 90 (Swiss prot) GI 32097, unnamed protein product [Homo sapiens]
 GI 119930610, PREDICTED: similar to Prostate, ovary, testis expressed protein on chromosome 2 [Homo sapiens]
 GI 88953571, PREDICTED: similar to Prostate, ovary, testis expressed protein on chromosome 2 isoform 2 [Homo sapiens]

Sample 3

RA Histone 4 (H4) (Swiss prot)
 GI 31645, glyceraldehyde-3-phosphate dehydrogenase [Homo sapiens] GI 28336, mutant beta-actin (beta'-actin) [Homo sapiens]
 GI 113413194, PREDICTED: similar to Prostate, ovary, testis expressed protein on chromosome 2 [Homo sapiens]
 GI 113413200, PREDICTED: similar to Prostate, ovary, testis expressed protein on chromosome 2 [Homo sapiens] GI 181967, elongation factor 1-alpha
 GI 1220311, elongation factor-1 alpha
 GI 31092, unnamed protein product [Homo sapiens] GI 178067, actin prepeptide
 GI 32486, unnamed protein product [Homo sapiens]
 GI 306891, 90kDa heat shock protein GI 32097, unnamed protein product (histone) [Homo sapiens]

Sample 4

RA Histone H4 (Swiss prot) Macrophage migration inhibitory factor (MIF) (Swiss prot)
Heat shock protein HSP 90-alpha (Swiss prot) Coiled-coil C2 domain-containing protein 1A (Swiss prot)
Actin, cytoplasmic 1 (Swiss prot) 14-3-3 protein zeta/delta (Swiss prot)
Histone H2B type 1-B (Swiss prot) L-lactate dehydrogenase A chain (Swiss prot)
Histone H2A type 1-B (Swiss prot)
Histone H3-like (Swiss prot) C4b binding protein alpha chain precursor (Swiss prot)
 GI 113413194, PREDICTED: similar to Prostate, ovary, testis expressed protein on chromosome 2 [Homo sapiens]
 GI 113413200, PREDICTED: similar to Prostate, ovary, testis expressed protein on chromosome 2 [Homo sapiens] GI 28336, mutant beta-actin (beta'-actin) [Homo sapiens]
 GI 32097, unnamed protein product (histone) [Homo sapiens]
 GI 88953571, PREDICTED: similar to Prostate, ovary, testis expressed protein on chromosome 2 isoform 2 [Homo sapiens]

Sample 5

Histone H4 (Swiss prot) Macrophage migration inhibitory factor (MIF) (Swiss prot)
Heat shock protein HSP 90-alpha (Swiss prot)
Elongation factor 1-alpha 1 (Swiss prot)
Peptidyl-prolyl cis-trans isomerase A (Swiss prot)
 GI 28336, mutant eta-actin (beta'-actin) [Homo sapiens]

Sample 6

Histone H4 (Swiss prot) Macrophage migration inhibitory factor (MIF) (Swiss prot)
Heat shock protein HSP 90-alpha (Swiss prot)
Elongation factor 1-alpha 1 (Swiss prot)
Peptidyl-prolyl cis-trans isomerase A (Swiss prot) Fatty acid synthase (Swiss prot)
 GI 17865718, Heat shock protein HSP 90-beta
 GI 113413194, PREDICTED: similar to Prostate, ovary, testis expressed protein on chromosome 2 [Homo sapiens]

Table 1. Proteins from three MCF7 samples of breast cancer cells treated with retinoic acid, Samples 2, 3, and 4. Untreated samples 1, 5, and 6. Note significant differences in the proteins in the treated samples compared to the untreated samples.