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13. ABSTRACT (Maximum 200 Words)

One of the earliest common changes in the development of breast cancer occurs when some breast epithelial cells begin to grow and proliferate independently of estradiol (E2). We have conducted studies on model breast epithelial cell lines: MCF7 cells, whose proliferation is dependent on E2; and LCC1 cells, a cell line derived from MCF7 cells with an acquired E2 independence for growth. We have continued to apply proteomics techniques (two-dimensional electrophoresis, image analysis and protein identification by mass spectrometry) to characterize broadly the patterns of protein expression in these two cell lines and their regulation by E2. We have identified several dozens of proteins in MCF7 cells which are up- of down-regulated in association with E2-controlled proliferation. Many of these are seen constitutively altered in the LCC1 cells grown in the absence of E2. While there is little or no effect of E2 in the proliferation of LCC1 cells, we find many proteins whose levels /are/ altered by the addition of E2. Our results are consistent with the hypothesis the E2-dependent MCF7 cells undergo apoptosis upon removal of E2 while the LCC1 cells have lost the ability to induce apoptosis upon removal of E2 and thereby disply E2-independent growth.

14. SUBJECT TERMS
Mass spectrometry, protein identification, 2D-gel electrophoresis, MCF7 cells, estrogen independent growth, diagnostic protein markers, protein targets for therapy, proteomics, cancer development

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INTRODUCTION

One of the earliest common changes in the development of breast cancer occurs when some breast epithelial cells begin to grow and proliferate independently of estradiol (E2). We have been conducting studies on model breast epithelial cell lines: MCF7 cells, whose proliferation is dependent on estradiol; and LCC1 cells, a cell line derived from MCF7 with an acquired E2 independence for growth. We continued to apply proteomics techniques (two dimensional electrophoresis, image analysis and protein identification by mass spectrometry) to characterize broadly the patterns of protein expression in these two cell lines and their regulation by estradiol. We have identified several dozens of proteins in MCF7 cells which are up- or down-regulated in association with E2-controlled proliferation. Many of these are seen constitutively altered in the LCC1 cells grown in the absence of E2. While there is little or no effect of E2 on the proliferation of LCC1 cells, we find many proteins whose levels are altered by the addition of E2. Our results are consistent with the hypothesis that E2-dependent MCF7 cells undergo apoptosis upon removal of E2 while the LCC1 cells have lost the ability to induce apoptosis upon removal of E2 and thereby display E2-independent growth.

BODY

Representative silver-stained 2D gels of total proteins isolated from MCF7 cells minus 17-β-estradiol (E2) covering three pH ranges are shown in Figure 1. Overlapping pH ranges are used to increase the number of considered protein features for a given complex sample. These images represent three of the approximately 500 such gels that have been through the course of this work. We have had good reproducibility in the gel images obtained for total cell proteins isolated from the various cell conditions tested: MCF7 cells plus or minus E2 and LCC1 cells plus or minus E2. Numerous attempts at subcellular fractionation and membrane isolation, while largely successful as judged by microscopy and 2D-gel based proteome analysis, were not sufficiently reproducible to allow precise analysis of differential protein expression.

Most of the initial time in this study was spent replicating Dr. Clarke's conditions as closely as possible by harvesting the cells 24 hours after addition of E2 to the medium. The effect of E2 on the proteomes of the MCF7 cells was virtually non-existent. That is to say the proteins detected in 2D gels from estrogen-stimulated cells were virtually indistinguishable from those of unstimulated cells. More consistent and numerous changes were observed when comparing MCF7 with LCC1 cells. As mentioned in the previous report (May 2001) we find a pronounced 1 day lag between the time of E2 addition and detection of increased growth of MCF7 cells. All studies in the present report were after re-stimulation of the cells with E2 for 48 hours whereby we observe many more significant and reproducible proteome changes (summarized below).

The switch from analyzing our gels using the Phoretix 2D gel analysis software (Nonlinear Dynamics) to using the more advanced Progenesis gel analysis software from the same vendor to take advantage of the enhanced spot detection, image warping, and database features of this new software. Even so, with our silver stained images, considerable time and effort was still required for spot editing and matching. We have completed the analysis of the pH 5-8 gels and have nearly completed the analysis of the pH 4-7, and 7-10 gel images. Spot detection is illustrated in Figure 2 and image warping and spot matching is illustrated in Figure 3. We use averaged gels constructed from multiple individual gels for a given condition. For a protein feature to be

considered it must appear and be matched in at least 4 out of 6 individual gels. For pH 5-8 gels, total number of spots satisfying this criterion for each condition are listed in Table 1. Table 2 summarizes the total protein changes observed upon addition of E2 to MCF7 and LCC1 cells and the effect of acquired E2 independent growth. Table 3 shows that there are very few E2-induced changes held in common between MCF7 and LCC1 cells whereas there are many changes held in common between the effect of E2 on MCF7 cells and the effect of acquired E2 independent growth (Table 4). The 73 proteins which increase (Tables 2 and 4) as a result of acquired E2 independent growth of LCC1 cells are potential markers for early detection of breast cancer. Preliminary analysis of the pH 7-10 gels shows comparatively few changes in protein expression induced by E2 or by the phenotype switch. As expected, analysis of the pH 4-7 gels confirms many of the changes summarized in Table 2-4 and add a few more acidic proteins. Summary statistics of the overlap and additional protein coverage afforded by these other pH ranges is not yet completed.

We have now identified most of the hundreds of protein alluded to in tables 2 through 4 by a combination of MALDI TOF MS peptide mass mapping and LC ESI MSMS analysis. The most consistent pattern emerging is a strong correlation with cell proliferation of proteins associated with polynucleotide synthesis and processing, protein synthesis, and nuclear transport. This is not surprising. There are also several changes observed in post-translationally modified protein species of nuclear lamins, several cytokeratins, and some chaperonins. One of the more interesting findings is that when E2 is removed from MCF7 cells and the cells cease to proliferate or even decrease in number, we find specific truncated forms of cytokeratins 18 and 19 appear (for example see Figures 4, 5 and 6). The full length cytokeratins 18 and 19 (not labeled) are found in the center left region of the gel (Figure 4) among the dark cluster of abundant poorly resolved proteins. Detailed sequence analysis using the mass spectrometry data suggests that all of these specific truncated forms are consistent with the action of pro-apoptotic caspases.

We examined further the idea that acquired estradiol independent growth in LCC1 cells results from a loss of activation of apoptosis upon removal of estradiol. The results of cell shakeoff and reattachment assays we have done are consistent with induction of apoptosis by removal of E2 from MCF7 but not LCC1 cells. More accurate flow cytometry experiments to determine the proportions of cells in G2/M phase, G1/G0, and cells with sub-G0 DNA content (indicative of apoptotic cells) for each of our four conditions (Figure 7). These results support the notion that E2-dependent MCF7 cells appear to leave S/G2/M phase and a fraction of them undergo apoptosis (as evidenced by the sub-G0 DNA content) upon removal of E2. The E2-independent LCC1 cells show a much smaller effect of removing E2 from the growth medium. To ask what pathways might be involved in regulating apoptosis we asked whether there were any changes in PARP cleavage products, indicators of induced cell death. We also asked whether apoptosis is constiuatively suppressed in LCC1 cells as opposed to not being so in MCF7 cells by looking at the phosphorylation status of Akt1. Many cell survival signals work by suppressing apoptosis through this pathway. The results of these experiments were inconclusive since we failed to find experimental conditions that gave good reproducible results by Western blotting techniques.

Complete summary statistics on protein changes in these experiments remain to becompiled for the manuscript being written for publication.

KEY RESEARCH ACCOMPLISHMENTS

Task 1. Set up 2D-electrophoresis system to analyze and compare the proteomes of MCF7 and MCF7/LCC1 breast cells and prepare initial 2D-gels for mass spectrometry protein identification. *(month 1-3)*

Completed in Year 1

Task 2. Implement software and techniques for producing a master gel pattern whereby changes in protein expression patterns among breast cell lines can be recognized with computer assistance. *(months 1-8)*

Completed in Year 1 and improved in year 2 (see body of report)

Task 3. Use mass spectrometry to sequence and identify remaining members of the set of abundant proteins that reflect the MCF7 response to estrogen and the MCF7/LCC1 acquisition of estrogen independence. *(months 2-4)*

Completed in year 2.

Task 4. Further elucidate the differences between MCF7 and MCF7/LCC1 cells and the response of these cells to estrogen to find those proteins that have eluded detection by virtue of their lower abundance *(months 6-18)* and/or previously unexplored isoelectric point range. *(months 9-21)*

Begun in year 1, extensive data collected in year 2, data collection completed in year 3, analysis is nearly complete..

Task 5. We will perform subcellular fractionation to characterize the proteomes of nuclear, soluble, and especially the membrane fractions. *(months 12-24)*

Begun in year 1, completed in year 2. This approach was judged too irreproducible for meaningful comparison of different cells grown under various conditions. The data are useful, however, for indicating which subcellular compartment a particular protein feature is associated with.

Task 6. Rapidly evolving improvements in mass spectrometry technology and database searching software will be implemented to improve sensitivity and to better sequence and identify newly selected proteins of lower abundance. *(months 1-24)*

We have continued to improve our general laboratory material handling practices allowing greater throughput and sensitivity in our mass spec analyses of protein features identified in gels. We have implemented a much more powerful image analysis package (Progenesis, Non-linear dynamics) than we were previously using. A new research grade MALDI TOF mass spectrometer (Reflex IV, Bruker Daltonics) was installed in year 2 allowing much more sensitive and accurate peptide mass mapping for protein identification. We have acquired and installed MASCOT (Matrix Science) for doing automated peptide mass map sequence database

searching on a local server. This has allowed higher throughput than using web-based search engines. MASCOT also provides a complement to our SEQUEST database searches of LC-MSMS data.

REPORTABLE OUTCOMES

Steven H. Seeholzer, Anthony T. Yeung, Bryan D. Oconnell, and Robert C. Clarke (2002). Proteomic analysis of Estradiol Independent Growth of a MCF7 Derivative Cell Line. *Era of Hope: Department of Defense Breast Cancer Research Program Meeting*, Orlando, Florida.

We shall presently finish the above reported work and prepare a manuscript for publication in a refereed journal. Remaining details to accomplish this task are to integrate the analyses of the overlapping pH renge gels with each other, prepare final figures, and confirm some of the more tentative protein identifications by mass spectrometry.

CONCLUSIONS

We have confirmed many of the expected E2-induced protein changes in MCF7 and LCC1 cells and have extended these observations to many newly identified protein markers associated with acquired estradiol independent growth. Estradiol appears to suppress apoptosis in dependent MCF7 cells whereas apoptosis appears to be constituitively suppressed in the estradiol-independent LCC1 cells.

REFERENCES

Skaar TC. Prasad SC. Sharareh S. Lippman ME. Brunner N. Clarke R. Two-dimensional gel electrophoresis analyses identify nucleophosmin as an estrogen regulated protein associated with acquired estrogen-independence in human breast cancer cells. Journal of Steroid Biochemistry & Molecular Biology. 67(5-6):391-402, 1998

Figures and Tables.

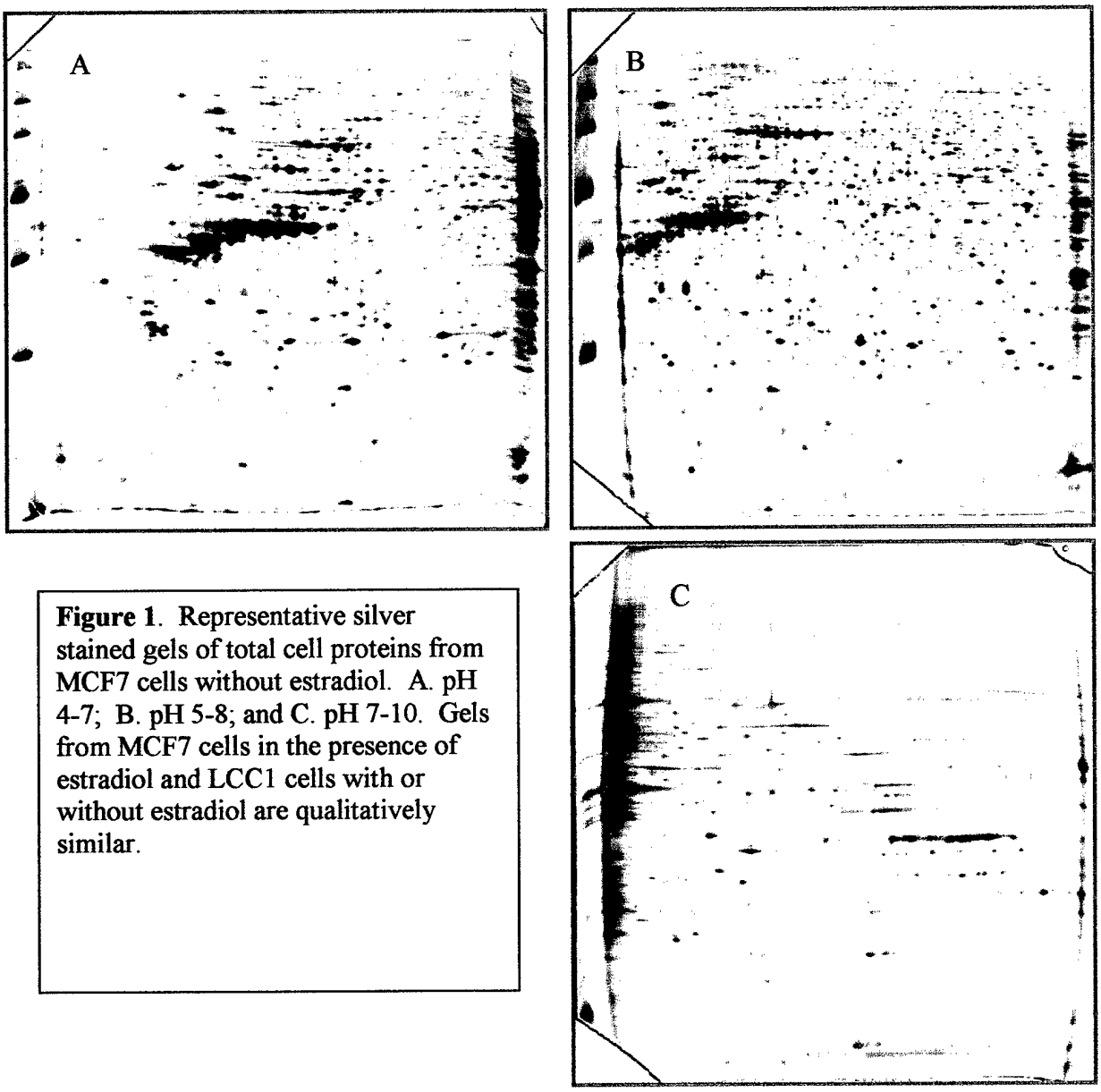

Figure 1. Representative silver stained gels of total cell proteins from MCF7 cells without estradiol. A. pH 4-7; B. pH 5-8; and C. pH 7-10. Gels from MCF7 cells in the presence of estradiol and LCC1 cells with or without estradiol are qualitatively similar.

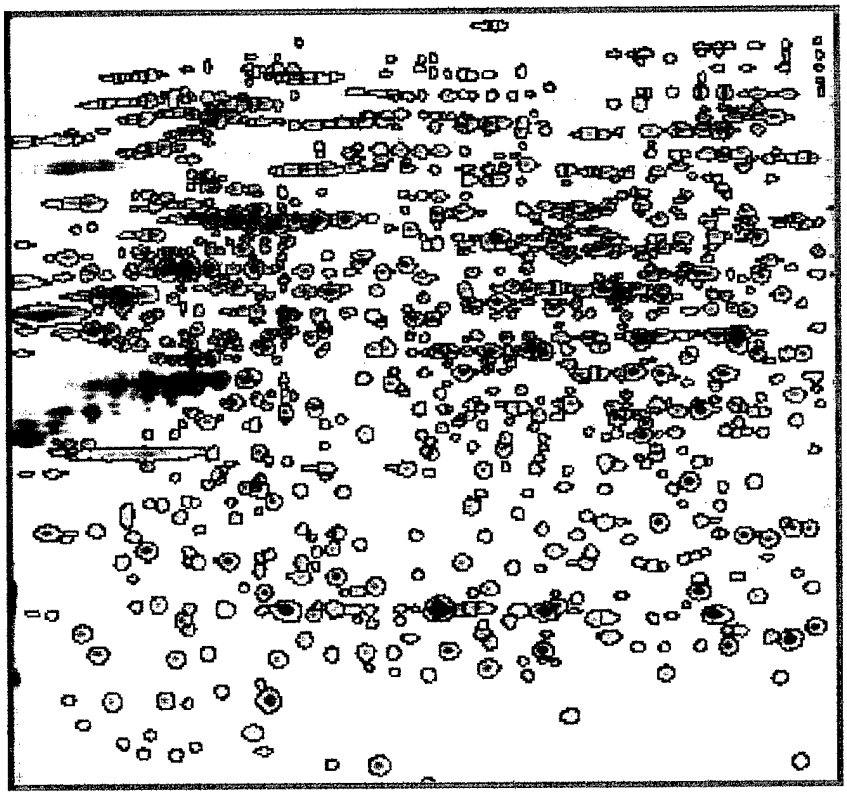

Figure 2. Illustration of spot detection by Progenesis software. Extensive editing of spot detection was found to be necessary in order to rejoin inappropriately split spots and split inappropriately joined spots.

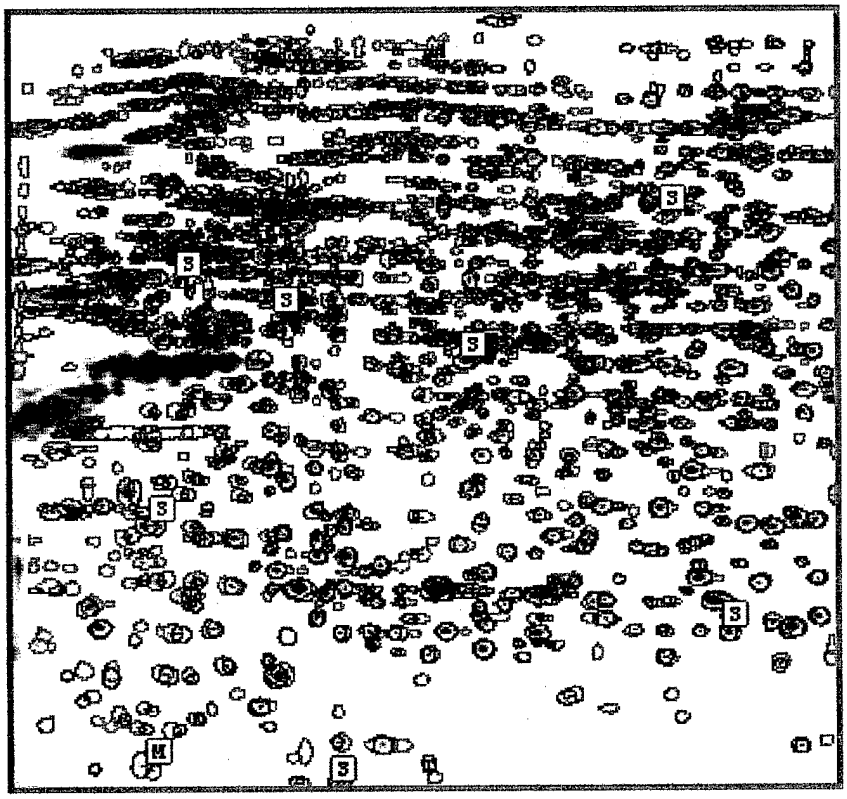

Figure 3. Illustration of gel image warping and spot matching procedure. User selected seeds for matching were chosen from highly reproducible local constellations of protein features. User seeds were invariably shown by mass spectrometry to be the same protein.

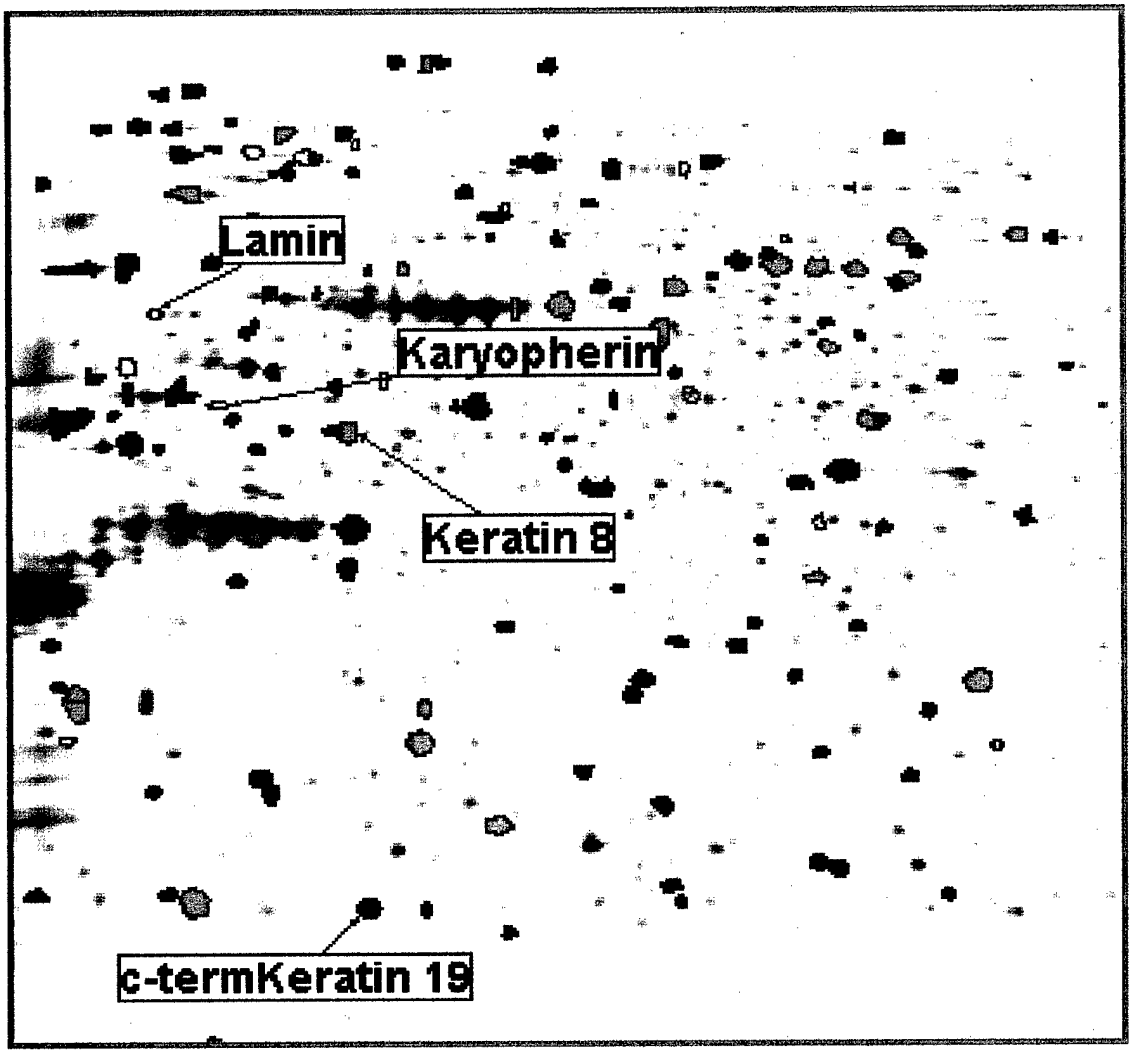

Figure 4. Gel image of MCF7 –E2 with E2-induced changes in MCF7 cells superimposed in color. Blue spots represent protein features not found in MCF7 +E2 but found in MCF7 –E2 (eg. C-term fragment of keratin 19). Green spots show a 2 fold increase upon removal of estradiol (eg Keratin 8) while yellow colored spots represent proteins decreased by the removal of estradiol (eg. Karyopherin, lamin).

Condition	Number of Unique Protein Features in Averaged Gels
MCF7 + E2	930
MCF7 - E2	814
LCC1 +E2	899
LCC1 –E2	891

Table 1. Summary of protein features comprising averaged gels for comparison across conditions. While 1200 to 1600 protein features were detected in each individual gel for each condition, these numbers represent protein features detected and matched in at least 4 out of every 6 gels for each condition.

Comparison	Increased	Decreased	Total change
MCF7 +E2 vs MCF7 – E2	45	29	74
LCC1 +E2 vs LCC1 – E2	13	47	60
LCC1 -E2 vs MCF7 – E2	73	51	124

Table 2. Effect of estradiol on MCF7 cells and on LCC1 cells. Shown are number of proteins showing greater than 4-fold changes in pH 5-8 2D gels.

Comparison	Increased	Decreased	Total change
MCF7 +E2 vs MCF7 – E2	44	26	70
Changes in common	1	3	4
LCC1 +E2 vs LCC1 – E2	12	44	56

Table 3. Effect of estradiol on MCF7 cells and on LCC1 cells. Shown are number of proteins with greater than 4-fold changes in pH 5-8 2D gels. The middle row shows those changes held in common between the two comparisons.

Comparison	Increased	Decreased	Total change
MCF7 +E2 vs MCF7 – E2	23	15	38
Changes in common	**22**	14	36
LCC1 –E2 vs MCF7 –E2	**51**	37	88

Table 4. Effect of estradiol on MCF7 cells and effect of acquired estradiol independence of LCC1 cells. Shown are number of proteins with greater than 4-fold changes in pH 5-8 2D gels.The middle row shows those changes held in common between the two comparisons.

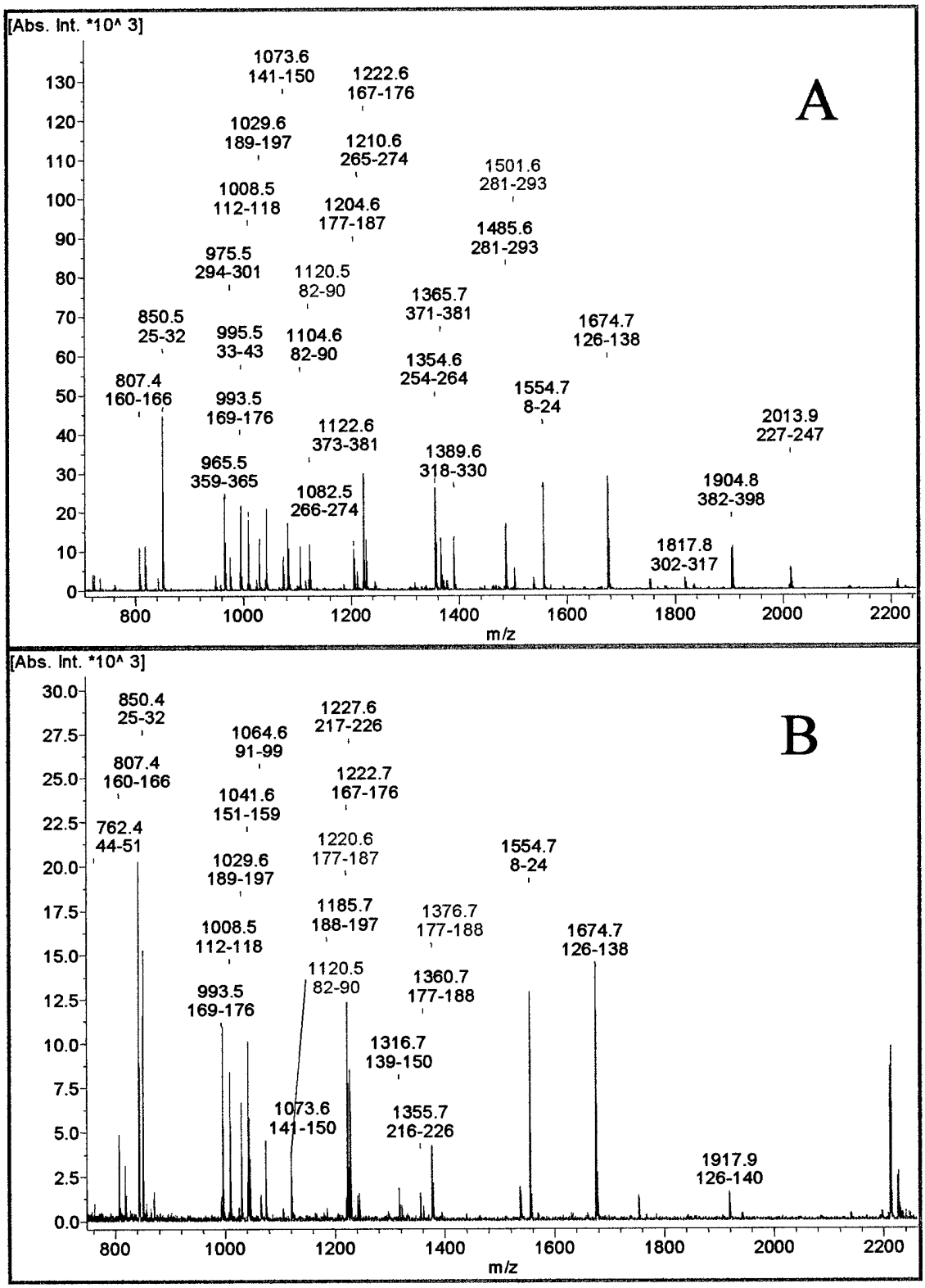

Figure 5. Tryptic peptide mass maps of full length (A) and proteolytically processed (B) keratin 19. The 2D gel spot giving rise to the processed form is only seen in MCF7 cells in the absence of estradiol (Figure 4).

Match to: **KRHU9**; Score: **338**
keratin 19, type I, cytoskeletal - human
Nominal mass (M_r): **44065**; Calculated pI value: **5.04**

A

Fixed modifications: Carbamidomethyl (C)
Variable modifications: Oxidation (M)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: **35**
Number of mass values matched: **26**
Sequence Coverage: **59%**

Matched peptides shown in **Bold Red**

1 MTSYSYRQSS **ATSSFGGLGG GSVRFGPGVA FRAPSIHGGS GGRGVSVSSA**
51 RFVSSSSSGG YGGGYGGVLT ASDGLLAGNE KLTMQNLNDR LASYLDKVRA
101 LEAANGELEV KIRDWYQKQG PGPSRDYSHY YTTIQDLRDK ILGATIENSR
151 IVLQIDNARL **AADDFRTKFE TEQALRMSVE ADINGLRRVL DELTLARTDL**
201 EMQIEGLKEE LAYLKKNHEE EISTLRGQVG GQVSVEVDSA PGTDLAKILS
251 DMRSQYEVMA EQNRKDAEAW FTSRTEELNR **EVAGHTEQLQ MSRSEVTDLR**
301 RTLQGLEIEL QSQLSMKAAL EDTLAETEAR FGAQLAHIQA LISGIEAQLG
351 DVRADSERQN QEYQRLMDIK SRLEQEIATY RSLLEGQEDH YNNLSASKVL
401

Match to: **KRHU9**; Score: **251**
keratin 19, type I, cytoskeletal - human
Nominal mass (M_r): **44065**; Calculated pI value: **5.04**

B

Fixed modifications: Carbamidomethyl (C)
Variable modifications: Oxidation (M)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: **46**
Number of mass values matched: **23**
Sequence Coverage: **44%**

Matched peptides shown in **Bold Red**

1 MTSYSYRQSS **ATSSFGGLGG GSVRFGPGVA FRAPSIHGGS GGRGVSVSSA**
51 **RFVSSSSSGG YGGGYGGVLT ASDGLLAGNE KLTMQNLNDR LASYLDKVRA**
101 LEAANGELEV KIRDWYQKQG PGPSRDYSHY YTTIQDLRDK ILGATIENSR
151 **IVLQIDNARL AADDFRTKFE TEQALRMSVE ADINGLRRVL DELTLARTDL**
201 EMQIEGLKEE LAYLKKNHEE **EISTLRGQVG** GQVSVEVDSA PGTDLAKILS
251 DMRSQYEVMA EQNRKDAEAW FTSRTEELNR EVAGHTEQLQ MSRSEVTDLR
301 RTLQGLEIEL QSQLSMKAAL EDTLAETEAR FGAQLAHIQA LISGIEAQLG
351 DVRADSERQN QEYQRLMDIK SRLEQEIATY RSLLEGQEDH YNNLSASKVL
401

Caspase consensus cleavage site: [ILV]ExD, shown in **Bold Blue**

Figure 6. Sequence coverage of full length (A) and proteolytically processed (B) keratin 19 corresponding to the peptide mass maps shown in Figure 5. The experimental pI and mass values match the calculated values in A but not in B.

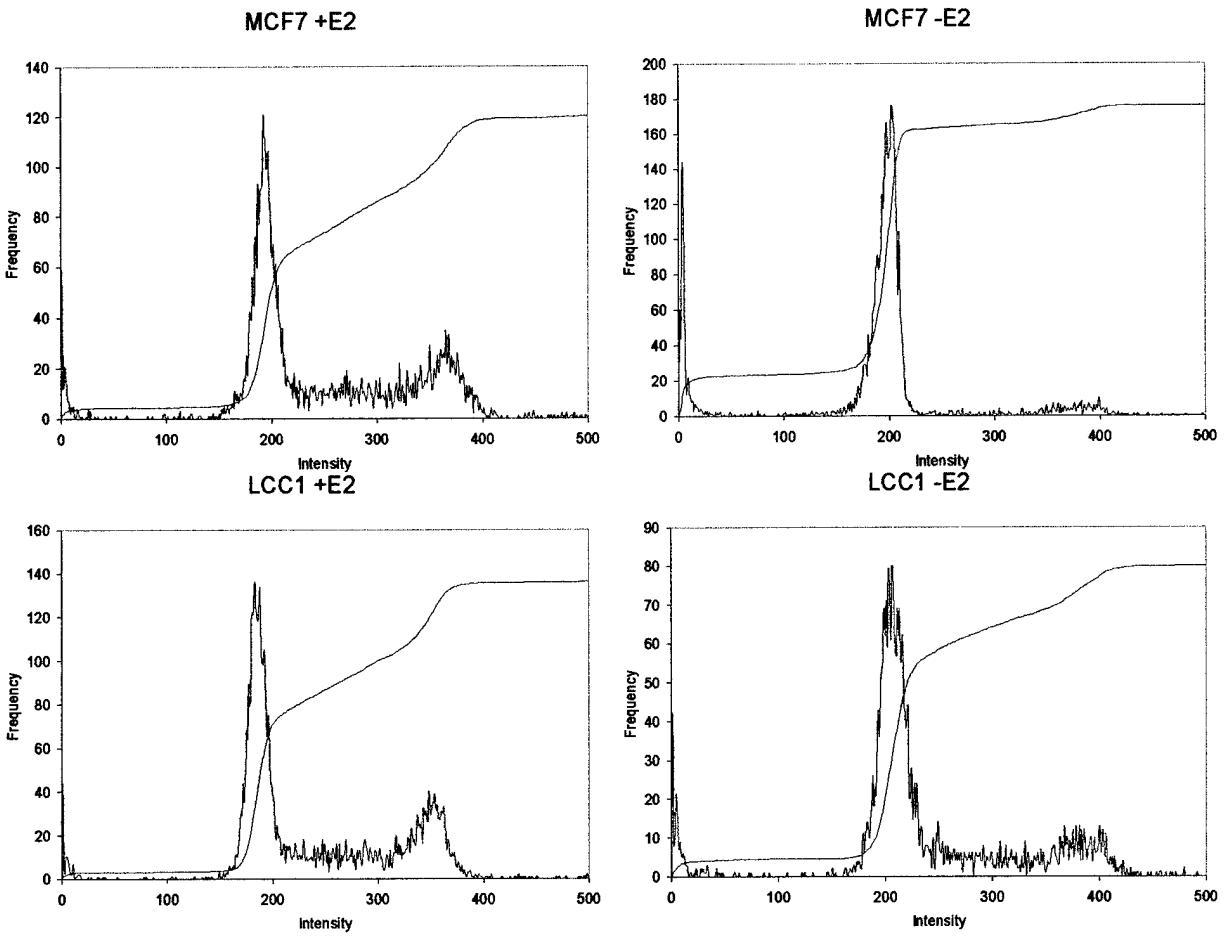

Figure 7. Effect of E_2 on mitotic index of MCF7 and LCC1 cells. Flow cytometry was used to measure DNA content of propydium iodide stained cells.

	FileName	Status	Score	Protein MW	Title	Coverage	
Increased in MCF7+E2, LCC1+or-E2					MCF7-E2 vs mCF7+E2 = MCF7-E2 vs ICC1-E2		
1	D:\BrukerNewData\0204\S0S\Sos01\1SF	Identified (multipl	93	94001.87	HSU46838 NID: - Homo sapiens	13	62
2	D:\BrukerNewData\0204\S0S\Sos02\1SF	Identified (multipl	184	94001.87	HSU46838 NID: - Homo sapiens	22	61
3	D:\BrukerNewData\0204\S0S\Sos03\1SF	Identified (multipl	117	86417.85	CDNA FLJ12466 FIS, CLONE NT2RM1000826, HIGHLY SIMI	19	60
4	D:\BrukerNewData\0204\S0S\Sos04\1Re	Undefined	77	100692.93	alpha-catenin 1 - human	11	59
5	D:\BrukerNewData\0204\S0S\SOS05b\1\	Identified (multipl	365	81856.93	replication licensing factor MCM7 - human	53	58
5	D:\BrukerNewData\0204\S0S\SOS05a\1\	Identified (multipl	400	81856.93	replication licensing factor MCM7 - human	61	57
6	D:\BrukerNewData\0204\S0S\SOS06b\1\		108	59720.01	keratin 10, type I, cytoskeletal - human	23	56
6	D:\BrukerNewData\0204\S0S\SOS06a\2\	Identified (multipl	153	59720.01	keratin 10, type I, cytoskeletal - human	33	55
7	D:\BrukerNewData\0204\S0S\Sos07\1SF		87	64938.57	HSU37436 NID: - Homo sapiens	22	54
8	D:\BrukerNewData\0204\S0S\Sos08\1SF	Identified (multipl	493	66521.75	LAMIN B1.- Homo sapiens (Human).	57	53
9	D:\BrukerNewData\0204\S0S\Sos09\1SF	Identified (multipl	154	58168.15	nuclear localization sequence receptor SRP1 alpha - human	48	52
10	D:\BrukerNewData\0204\S0S\SOS10\1S	Identified (multipl	137	46751	CDNA FLJ20570 FIS, CLONE REC00956 (FRAGMENT).- Ho	40	51
11	D:\BrukerNewData\0204\S0S\SOS11\1R		109	59720.01	keratin 10, type I, cytoskeletal - human	28	50
12	D:\BrukerNewData\0204\S0S\SOS12\1S	Identified (multipl	240	51087.15	HSY13286 NID: - Homo sapiens	57	49
13	D:\BrukerNewData\0204\S0S\SOS13\1R		83	46979.26	ribonucleoprotein La - human	25	48
14	D:\BrukerNewData\0204\S0S\SOS14\1R	Identified (multipl	180	38624.27	MEMBRANE ASSOCIATED PROTEIN SLP-2 (STOMATIN-LI	47	47
15	D:\BrukerNewData\0204\S0S\SOS15\1S	Undefined	61	47890.31	DJ657E11.4 (SIMILAR TO 60S ACIDIC RIBOSOMAL PROTEI	22	46
16	D:\BrukerNewData\0204\S0S\SOS16\1S	Identified (multipl	118	37946.43	arfaptin 2 - human	36	45
17	D:\BrukerNewData\0204\S0S\SOS17\1S		73	36383.55	4921530D09RIK PROTEIN.- Mus musculus (Mouse).	30	44
18	D:\BrukerNewData\0204\S0S\SOS18\1S	Identified (multipl	184	30683.15	UNKNOWN (PROTEIN FOR MGC:10739) (SIMILAR TO HET	48	43
19	D:\BrukerNewData\0204\S0S\SOS19\1S	Identified (multipl	154	34991.79	AF000576 NID: - Rattus norvegicus	47	42
20	D:\BrukerNewData\0204\S0S\SOS20\1S	Identified (multipl	126	40920.89	CGI-52 PROTEIN.- Homo sapiens (Human).	39	41
21	D:\BrukerNewData\0204\S0S\SOS21\1S	Identified (multipl	180	38757.35	HUMPOLACCA NID: - Homo sapiens	56	40
22	D:\BrukerNewData\0204\S0S\SOS22\1S	Undefined	64	28850.02	HUMRPS6A NID: - Homo sapiens	26	39
23	D:\BrukerNewData\0204\S0S\SOS23\2S	Identified (multipl	214	33635.97	thiosulfate sulfurtransferase (EC 2.8.1.1) - human	52	38
24	D:\BrukerNewData\0204\S0S\SOS24\2R	Identified (multipl	128	17688.21	HSBTF3 NID: - Homo sapiens	62	37
25	D:\BrukerNewData\0204\S0S\SOS25\1S	Identified (multipl	112	17908.03	dUTP pyrophosphatase (EC 3.6.1.23) - human	65	36
26	D:\BrukerNewData\0204\S0S\SOS26\1R		90	21541.68	HYPOTHETICAL 21.5 KDA PROTEIN.- Homo sapiens (Huma	51	35
27	D:\BrukerNewData\0204\S0S\SOS27\1S	Undefined	62	19910.86	*modifier protein 2 - mouse*	31	34
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30	D:\BrukerNewData\0204\S0S\SOS30\1R	Identified (multipl	264	61187.48	*chaperonin GroEL precursor - human*	51	31
31	D:\BrukerNewData\0204\S0S\SOS31\1S	Identified (multipl	255	50804.01	*tubulin alpha-1 chain - Chinese hamster*	63	30
32	D:\BrukerNewData\0204\S0S\SOS32\1S	Identified (multipl	174	56318.48	*ATP SYNTHASE BETA CHAIN, MITOCHONDRIAL PRECUF*	48	29
33	D:\BrukerNewData\0204\S0S\SOS33\1S	Identified (multipl	271	50095.24	*tubulin beta-7 chain - chicken*	69	28
34	D:\BrukerNewData\0204\S0S\SOS34\1S	Identified (multipl	304	36243.5	EUKARYOTIC TRANSLATION INITIATION FACTOR 2 ALPHA	67	27
35	D:\BrukerNewData\0204\S0S\SOS35\2S		110	36031.42	*UNKNOWN (PROTEIN FOR MGC:4272).- Homo sapiens (Hu*	32	26
36	D:\BrukerNewData\0204\S0S\SOS36\1S	Identified (multipl	265	47305.2	*HSCYKT18 NID: - Homo sapiens*	39	25
37	D:\BrukerNewData\0204\S0S\SOS37\1R	Identified (multipl	269	42386.04	*MMEIF4AI NID: - Mus musculus*	53	24
Increased in -E2							
1	D:\BrukerNewData\0204\S0S\SOS38\2S	Identified (multipl	144	51229.57	dC stretch-binding protein CSBP - rat	39	23
2	D:\BrukerNewData\0204\S0S\SOS39\1S	Identified (multipl	141	51229.57	dC stretch-binding protein CSBP - rat	33	22
3	D:\BrukerNewData\0204\S0S\SOS40\1S	Identified (multipl	127	106126.31	SEQUENCE 5 FROM PATENT WO9724448.- unidentified.	19	21
4	D:\BrukerNewData\0204\S0S\SOS41\1S	Identified (multipl	125	74539.76	KIAA1499 PROTEIN (FRAGMENT).- Homo sapiens (Human).	28	20
5	D:\BrukerNewData\0204\S0S\SOS42\1S	Identified (multipl	157	59720.01	keratin 10, type I, cytoskeletal - human	34	19
6	D:\BrukerNewData\0204\S0S\SOS43\1S	Identified (multipl	531	65152.62	lamin C - human	66	18
7	D:\BrukerNewData\0204\S0S\SOS44\1S	Identified (multipl	444	74379.88	lamin A - human	54	17
8	D:\BrukerNewData\0204\S0S\SOS45\1S		106	56744.26	catalase (EC 1.11.1.6), chain D - human	31	16
9	D:\BrukerNewData\0204\S0S\SOS46\1S		107	55673.65	HSA007702 NID: - Homo sapiens	32	15
10	D:\BrukerNewData\0204\S0S\SOS47\1R	Identified (multipl	186	53529.03	HUMDKERB NID: - Homo sapiens	29	14
11	D:\BrukerNewData\0204\S0S\SOS48\1S	Identified (multipl	412	53529.03	HUMDKERB NID: - Homo sapiens	62	13
12	D:\BrukerNewData\0204\S0S\SOS49\2R	Identified (multipl	335	53529.03	HUMDKERB NID: - Homo sapiens	59	12
13	D:\BrukerNewData\0204\S0S\SOS50\1R	Identified (multipl	497	53529.03	HUMDKERB NID: - Homo sapiens	63	11
14	D:\BrukerNewData\0204\S0S\SOS51\1S	Identified (multipl	251	37849.68	HSA9985 NID: - Homo sapiens	66	10
15	D:\BrukerNewData\0204\S0S\SOS52\1S	Undefined	64	33746	T-CELL RECEPTOR ALPHA CHAIN.- Mus musculus (Mouse	24	9
16	D:\BrukerNewData\0204\S0S\SOS53\1S	Identified (multipl	223	36392.72	annexin iii - human	54	8
17	D:\BrukerNewData\0204\S0S\SOS54\1R	Identified (multipl	149	37687.56	HSTALDR3 NID: - Homo sapiens	34	7
18	D:\BrukerNewData\0204\S0S\SOS55\1S	Identified (multipl	165	28468.83	calpain (EC 3.4.22.17) small chain - human	42	6

Table 5. Partial list of E_2 regulated proteins in MCF7 cells and proteins constitutively regulated in LCC1 cells. The upper and lower groups represent proteins upregulated or downregulated, respectively, by E_2 in MCF7 cells or constitutively so regulated in LCC1 cells. Those entries shown in red in the sixth column are held in common when comparing the E_2 effect on MCF7 cells with the effect of phenotype switch between MCF7 and LCC1 cells.

LIST OF PERSONNEL

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