Protein content study revealed presence of isoform 2 of beta-tropomyosin in primary breast cancer tissues from Sudanese patients

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Abstract:

This study was designed to compare antigen content of normal and cancerous breast tissues of Sudanese patients.

Methods: 50 tissue samples- normal and cancerous - from 25 Sudanese patients with primary breast cancer were analyzed for their protein content using 2D PAGE and for protein identification using LC/MS and nr.fasta data base search.

Results: Beta-Tropomyosin spot was found in all the cancerous tissues and absent from all the normal tissues of the same patients. The protein is isoform 2 with 257 amino acids.

Conclusion: Primary breast cancer tissues from Sudanese patients are characterized by the presence of isoform 2 of beta-tropomyosin, which is not detected in the normal tissues.

Key words: Primary breast cancer, Beta-tropomyosin,2D PAGE ,LC/MS, nr.fasta

Introduction

Breast cancer is the highest prevalent cancer among females in east Africa region (34.5%)[1]. An epidemiological study in the Sudan showed that breast cancer is the most prevalent malignancy in females. However it has been reported that it is rarely seen in women below 30 years of age ($p = 0.006$)[1].One in ten of all new cancers diagnosed worldwide is a cancer of the female breast in both developing and developed countries. It is also the principal cause of death from cancer among women globally[2]. Breast cancer is characterized by abnormal antigens which can be detected and characterized.

Large scale proteomics plays a critical role in the rapid display, identification and validation of new target antigens. These new antigens can be used for the study of the disease development, progression and severity[3].

Tropomyosin is one of the abnormally expressed proteins in breast cancer[3]. It is composed of two polypeptide chains, alpha (TPM1, TPM3 and TPM4) and beta (TPM2). The beta-tropomyosin (TPM2) has three isoforms; isoform-1 with 284 amino acids, isoform- 2 with 284 amino acids and isoform- 3 with 248 amino acids[4-6]. Beta- Tropomyosin binds actin filaments in muscle and non muscle cells. It plays a central role in association with the troponin complex, in the calcium dependant regulation of vertebrate striated muscle contraction. Smooth muscle contraction is regulated by interaction with caldesmon. In non muscle cells, beta-tropomyosin is implicated in stabilizing cytoskeleton actin filaments[7].

There are two diseases associated with defects in beta-tropomyosin. Nemaline Myopathy type 4 (NEM4) is a form of congenital myopathy characterized by abnormal thread or rod like structures in muscle fibers on histologic examination[8].The second disease is the Distal Arthrogryoposis type1(DA1). DA1 is a form of inherited multiple congenital contractures[9].

This study was designed to compare the protein content of normal breast tissues with the primary cancerous breast tissues of Sudanese patients. The emerging information can be used as a tool for diagnosis, treatment and even prevention of breast cancer.

Material and Methods

Malignant and normal tissues were obtained after informed consent from 25 patients histologically confirmed to have primary breast cancer. Tissue samples were taken from the tumour and from the macroscopically normal tissue and transferred directly to liquid nitrogen.

Sample preparation

Tissue samples were first grinded using liquid nitrogen cooled mortar and pestle and homogenized by addition of 10 ml of lysis buffer , which is composed of 7 M Urea, 4% Chaps, 2M Thiourea and 30 mM Tris , the pH is adjusted to 8.5. The sample solutions were then centrifugated at 13000g for 15 minutes at 4°C , then cleaned 10 times by Ettan™ Sample preparation kits and reagents 2D Clean-Up kit (Amersham, UK) The protein concentration in the supernatent was determined using Bio-Rad protein assay kit with BSA as standard . The supernatant was kept at -80°C.

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Analytical 2D PAGE

Isoelectric focusing (IEF) was performed on immobilized pH- linear gradient (IPG, pH 3-10, 18 cm) with Immobline™ Dry Strip (Amersham, UK). 400µg of samples were loaded directly on the strips and rehydrated for 12 hours at room temperature. Focusing was performed with 50µA per strip at 20°C with 4 step voltage modes, Step and Hold - 500V , 0.5 kV/ h -, Gradient (1000 V, 0.8 kV/ h), Gradient (8000 V, 13.5 kV/ h) and Step and Hold and Hold (8000 V,12.2 kV/h).

Separation of the second dimension was performed in 12.5% SDS/ Polyacrylamide gel (25 × 20 cm) using the Hofer DALT System six electrophoresis unit (Amersham, UK). Proteins in the analytical gel were stained with Coomassie stain. The Coomassie stain was prepared using Coomassie Brilliant Blue G-250 stock following the manufacturer's protocol.

Gel analysis

The stained gel was examined by image scanner II (Amersham, UK) using LabScan5 software (Amersham, UK). The interested spot was digested by the Trypsin enzyme using Ettan™ Spot Handling Workstation (Amersham, UK). After digestion, the interested spot was analyzed by LC/MS/MS followed by database comparison.

Liquid Chromatography and Mass Spectroscopy

A total of 5µl of protein digest was load onto a reversed-phase(RP) C18 column for LC/MS/MS analysis. The HPLC system used was Finnigan Surveyor™ MS pump with a flow splitter, column 0.18×100 mm C18 (Thermo Electron, USA) and flow rate 200ul/min while the mobile phases were A: Water with 0.1% Formic Acid and B: Acetonitrile with 0.1%Formic acid and gradients were 2-60% B in 20 min , 65-80 % B in 5 min , hold 5 min and 80-2% B in 2 min.

LC/MS/MS used was Finnigan LTQ Linear Ion Trap Mass Spectrometer (Thermo Electron, USA) and the condition were as follows : Ionization mode was NanoSpray, positive ion, 200° C Capillary temperature, 1.8 KV Spray needle voltage, 400-1,600 m/z Mass range, Scan sequence was Full-scan MS, MS/MS scan and Acquisition modes were Normal, Data Dependent and Dynamic Exclusion.

Data Analysis

Protein identification was performed using the Turbo SEQUEST algorithm in the BioWorksTM3.1SR1 software package (Thermo Electron, USA) and nr.fasta database.

Results

Using the 2D-PAGE many protein spots were visualized within the pH range of 3-10 and a molecular mass range of 10 to 200 kD (Fig.1A and Fig.1B).

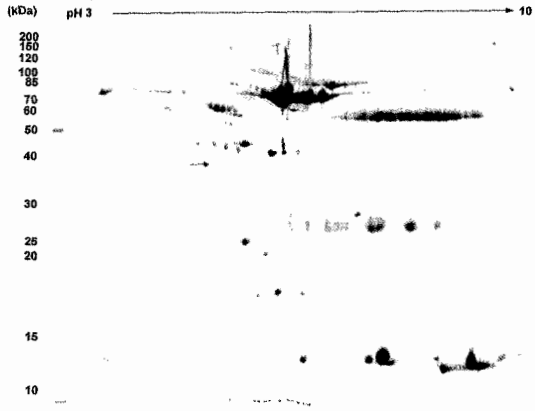


Fig.1A: 2D PAGE result of normal breast tissue.

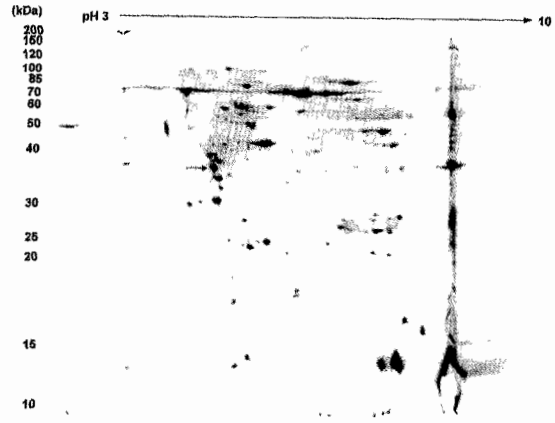
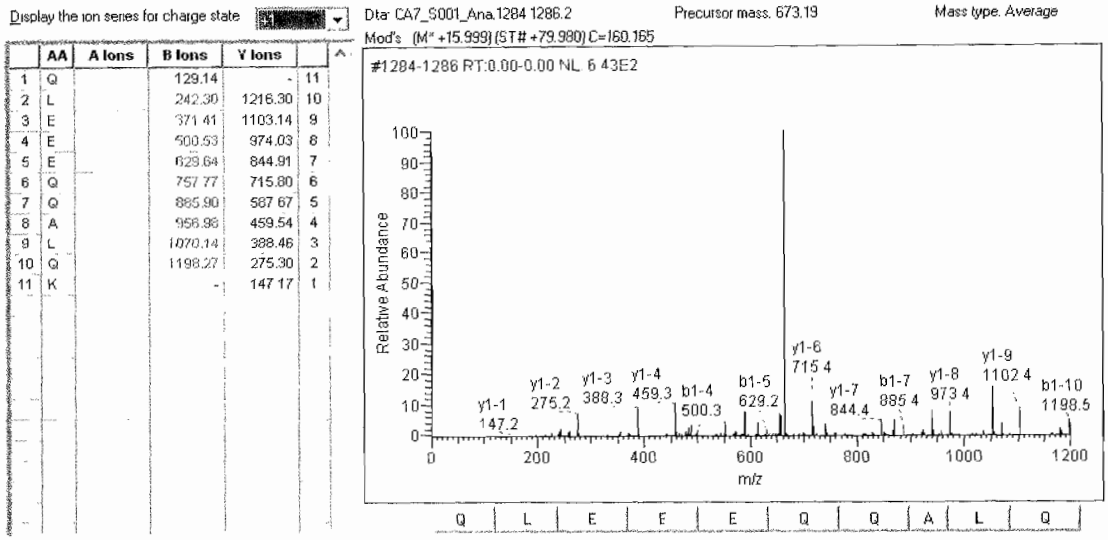


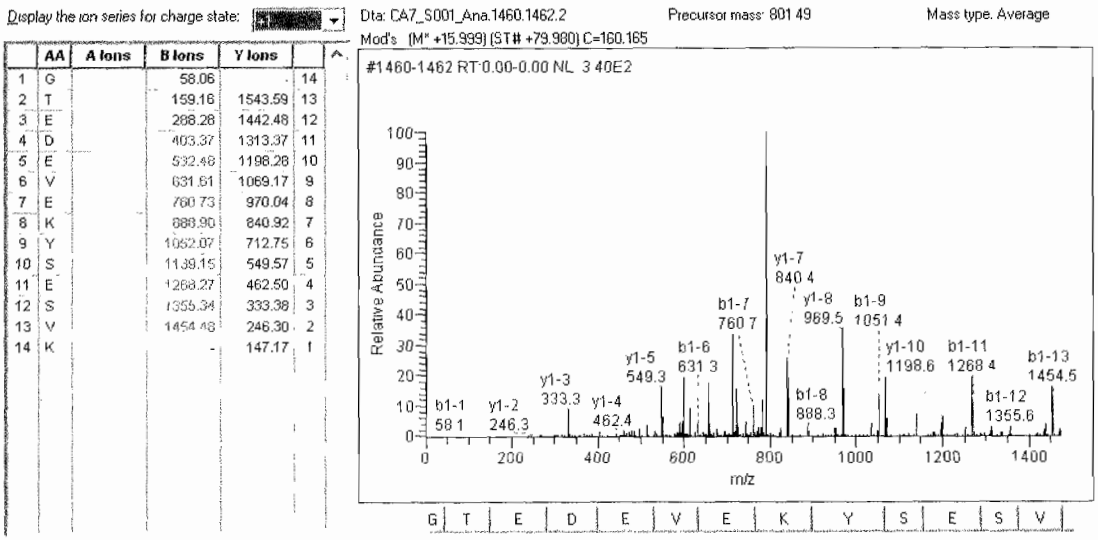
Fig.1B: 2D PAGE result of primary cancerous tissue.
Representative 2D PAGE map of normal A and primary cancerous B breast tissues 400 microgram samples were separated by 2D PAGE using 18 cm pH 3- 10 linear strip and 12.5% SDS-PAGE. Proteins were detected by coomassie blue stain. The stained gels were examined by image scanner II using LabScan5 software (Amersham, UK).

The protein profiles of the matched normal and tumour samples were not identical. Nine spots were absent from all the normal samples but present in all the tumour samples. One of the nine abnormal spots was characterized using LC/MS and nr.fasta database search and was identified as isoform 2 of beta-tropomyosin (Fig.1B , and App.1, App.2 and App.3). The beta-tropomyosin was composed of 257 amino acids, with iso-electric point of 4.70 and average mass of 29942.3 (Fig.2)



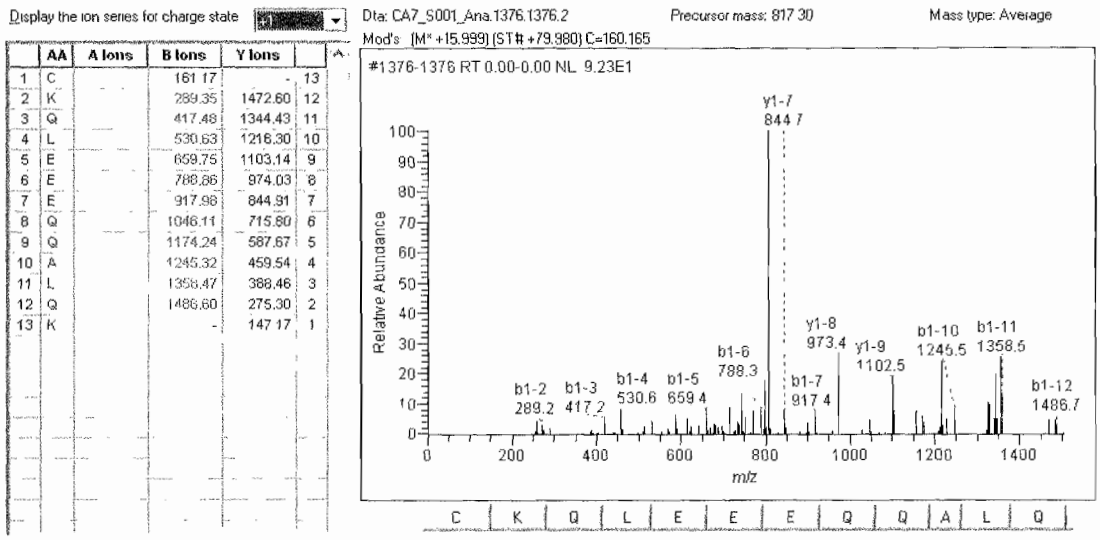
	AA	A Ions	B Ions	Y Ions	
1	Q		129.14	-	11
2	L		242.30	1216.30	10
3	E		371.41	1103.14	9
4	E		500.53	974.03	8
5	E		629.64	844.91	7
6	Q		757.77	715.80	6
7	Q		885.90	587.67	5
8	A		956.96	459.54	4
9	L		1070.14	388.46	3
10	Q		1198.27	275.30	2
11	K		-	147.17	1

App.1 : Fragment ion of precursor mass 673.19 of beta-tropomyosin.



	AA	A Ions	B Ions	Y Ions	
1	G		58.06	-	14
2	T		159.16	1543.59	13
3	E		288.28	1442.48	12
4	D		403.37	1313.37	11
5	E		532.48	1198.28	10
6	V		631.61	1069.17	9
7	E		760.73	970.04	8
8	K		888.90	840.92	7
9	Y		1052.07	712.75	6
10	S		1139.15	549.57	5
11	E		1268.27	462.50	4
12	S		1355.34	333.38	3
13	V		1454.48	246.30	2
14	K		-	147.17	1

App.2 : Fragment ion of precursor mass 801.49 of beta-tropomyosin



	AA	A Ions	B Ions	Y Ions	
1	C		161.17	-	13
2	K		289.35	1472.60	12
3	Q		417.48	1344.43	11
4	L		530.63	1216.30	10
5	E		659.75	1103.14	9
6	E		788.86	974.03	8
7	E		917.98	844.91	7
8	Q		1046.11	715.80	6
9	Q		1174.24	587.67	5
10	A		1245.32	459.54	4
11	L		1358.47	388.46	3
12	Q		1486.60	275.30	2
13	K		-	147.17	1

App.3 : Fragment ion of precursor mass 817.30 of beta-tropomyosin

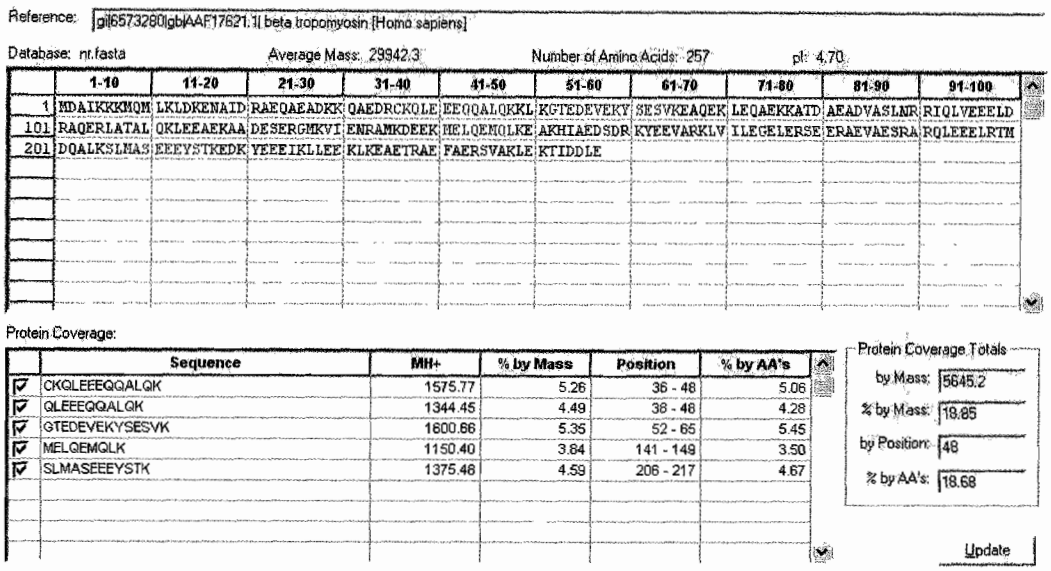


Fig.2:nr.fasta spectrum identification result.
The search result showing the amino acid sequence, number of amino acids, Average mass and the Isoelectric point. The red colored sequences are the spectrum sequences (sites of enzyme digestion). This result was obtained after sending the spectrums (App.1, App.2 and App.3) of the LC/MS.

Discussion

Presence of tropomyosin-1 was reported previously in benign tumours, primary cancer tissue and in normal human mammary epithelial cell line[10-13]. High levels of beta- tropomyosin in progressive breast cancer cell line were reported[14-15]. Down regulation of the tropomyosin molecule isoforms expression in primary breast tumor cell line was mentioned in the literature[10,11]. Presence of beta-tropomyosin in primary breast cancer tissues may play a role in the metastasis of tumor cells[14, 15].

Our study is a qualitative study. We have investigated the presence or absence of beta-tropomyosin spot in cancerous and normal breast tissues without determining the level.

Going with other studies we found that primary breast cancer tissues are associated with the presence of isoform2 of beta-tropomyosin[14,15]. However, we could not detect this protein in normal breast tissues of Sudanese patients. This finding is probably unique one. We could not find a similar report in the English published literature. It seems that this is the first time to register that the isoform2 of beta-tropomyosin is undetectable in normal breast tissues. This new finding may advocate and augment trials of producing breast cancer vaccine.

Conclusion

Isoform 2 of beta-tropomyosin is characteristically undetectable in normal Sudanese breast tissues, while it is present in cancerous tissues.

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