doi: 10.4149/gpb_2016009

Short Communication

A comparative study of protein patterns of human estrogen receptor positive (MCF-7) and negative (MDA-MB-231) breast cancer cell lines

Dana Flodrova[1], Lucia Toporova[2], Dana Macejova[2], Marketa Lastovickova[1], Julius Brtko[2] and Janette Bobalova[1]

[1] *Institute of Analytical Chemistry of the CAS, v. v. i., Veveří 97, 602 00 Brno, Czech Republic*
[2] *Institute of Experimental Endocrinology, BMC, Slovak Academy of Sciences, Dúbravská cesta 9, 845 05 Bratislava, Slovak Republic*

Abstract. In the present study, we analyzed the cell lysates of human tumour cell lines representing two major clinically different types of breast cancer. Our main goal was to show the differences between them on proteomic level. Gel electrophoresis followed by MALDI-TOF MS analysis was used for proteins determination. Exactly 98 proteins were unequivocally identified and 60 of them were expressed differentially between MDA-MB-231 and MCF-7 cell lines. Among the proteins reported here, some well-known breast cancer markers (e.g., annexin A1, annexin A2 and vimentin) were identified in the MDA-MB-231 cell line and thus we were able to distinguish both cell lines sufficiently.

Key words: Cell line — Breast cancer — Mass spectrometry — Proteins

In the present cancer research, tumour cell lines have wide utilization as *in vitro* models that provide essential tools in many aspects of laboratory research. Their using offers a lot of advantages, e.g., unlimited self-replication source of sample, easy handling, and relatively high degree of homogeneity (Khan 2013; Bialesova et al. 2015). Nevertheless, the utilization of cell lines has also some drawbacks because they do not represent the current diversity and heterogeneity of tumour, and moreover their genotype and phenotype can drift during their continual culture (Mladkova et al. 2010).

Generally, breast cancers are assorted into 3 clinical types based on the presence or absence of three biomarkers: (1) human epidermal growth factor receptor 2 (HER2)-positive, (2) estrogen receptor (ER) and/or progesterone (PR)-positive/HER2-negative and (3) triple-negative (Whelan et al. 2012). MCF-7, established at the Michigan Cancer Foundation in 1973 (Soule et al. 1976), and MDA-MB-231 established in 1974 (Cailleau et al. 1974) are the most commonly used breast cancer cell lines in the world. Both lines originated from pleural effusions of metastatic mammary carcinoma patients (Mladkova et al. 2010). MCF-7 cells were isolated from 69 year old woman with metastatic disease (Keen 2010) and they represent an ideal model for studies of hormone response because they have high hormone sensitivity through expression of ER (Levenson and Jordan 1997). This cell line expresses markers of the luminal epithelial phenotype of breast cells, the MDA-MB-231 line does not express them but it contains a high level of vimentin, a marker of the mesenchymal phenotype. MDA-MB-231 is also used as a model for triple negative breast cancers (Mladkova et al. 2010). These two cell lines are used in the present study in an attempt to compare their protein patterns by using the combination of two methodological approaches, SDS-PAGE and mass spectrometry (MS).

The cancer cell cultures were purchased from the HPACC (Salisbury, Great Britain). Cells were grown and passaged routinely as monolayer cultures. For experiments, the cells were used at passage 10–30. Cells were seeded in Petri dishes in Dulbecco´s modified Eagle´s medium (DMEM) supplemented with 10% fetal bovine serum (FBS), antibiotics (penicillin, streptomycin, gentamicin) and cultured in humidified atmosphere of 5% CO_2 and 95% air at 37°C. After incubation, the cells were washed with ice-cooled PBS. The cell lysis was made according to manual instruction from the

Correspondence to: Janette Bobalova, Institute of Analytical Chemistry of the CAS, v. v. i., Veveří 97, 602 00 Brno, Czech Republic
E-mail: bobalova@iach.cz

RIPA buffer by Sigma. The cell lysates were stored at –70°C for further use. Before gel electrophoresis, samples were dialyzed against deionized water using Slide-A-Lyzer dialysis cassettes (Thermo Fisher Scientific, Waltham, MA, USA) with 2 kDa cut-off and lyophilized. Purified samples were dissolved in 300 µl of the Laemmli sample buffer. After briefly being boiled (10 min, 95°C) in a water bath, samples were applied onto the 12% SDS gel. Separations were performed at constant voltage 140 V. The visualization was carried out using Coomassie Brilliant Blue G-250 dye. Stained protein spots were excised from the gel and digested (after reduction with 10 mM dithiothreitol and subsequent alkylation with 55 mM iodoacetamide) with trypsin (digestion buffer: 50 mM NH_4HCO_3, 5 mM $CaCl_2$, 12.5 ng/µl of enzyme) overnight at 37°C. The resulting tryptic peptides were extracted from the gel by 0.1% trifluoroacetic acid (TFA) and acetonitrile (1:1, v/v). For mass spectrometric analyses, the extracts were purified by ZipTip C_{18} (Millipore).

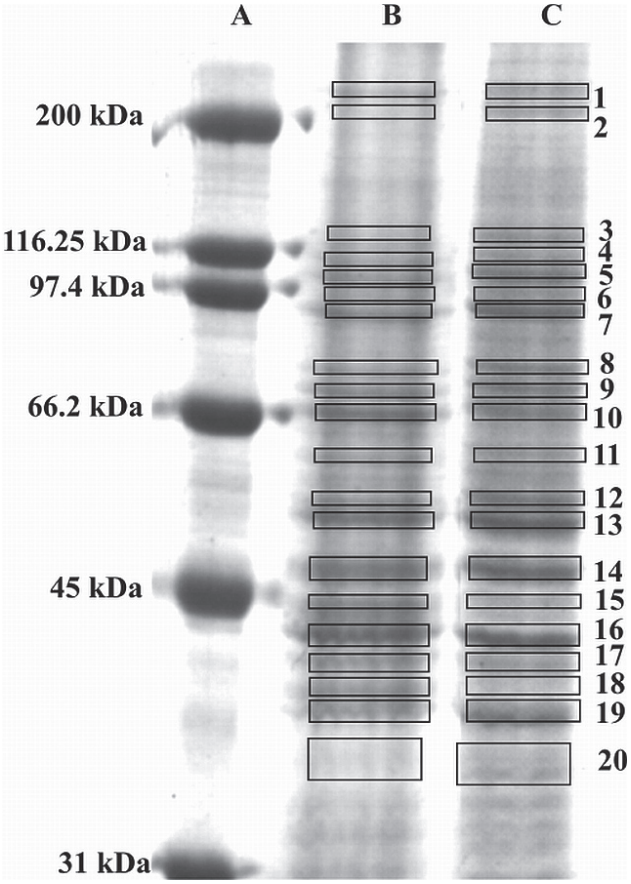

Figure 1. SDS-PAGE separation of cell lysis (molecular weight markers, **A**) from individual human breast cancer cell lines: MCF-7 ER^+ cell line (**B**) and MDA-MB-231 ER^- cell line (**C**). The rectangles mark excised and MS analyzed proteins bands which were successfully identified.

A solution of α-cyano-4-hydroxycinnamic acid (8 mg/ml in acetonitrile/0.1% TFA, 1:1, v/v) was used for both MS and MS/MS analysis of peptides. MALDI MS experiments in positive ion reflectron mode were performed on AB SCIEX TOF/TOF™ 5800 System (AB SCIEX, Framingham, MA, USA) equipped with a 1 kHz Nd:YAG laser. Acquired mass spectra were processed using 4000 Series Explorer software and the data were submitted to the Mascot database searching. Protein identifications were assigned using the NCBInr database with taxonomy restriction to *Homo sapiens*. Maximum tolerance for peptide masses as well as fragment error was set to 0.3 Da. Additional parameters used: enzyme trypsin; allowed missed cleavages: up to one, fixed modification: carbamidomethyl, no variable modification; peptide charge: +1; monoisotopic masses; instrument MALDI-TOF/TOF.

In this work, we compared protein patterns between MCF-7 cell line and MDA-MB-231 cell line by simply comparative proteomics based on combination of 1D-GE and tandem MS. Comparative proteomics offers a dynamic view on the proteome including the qualitative and quantitative changes of proteins (Farinha et al. 2011). Our study had two aims: a) to perform a basic comparison of breast cell lines based on SDS-gels protein profiles; and b) to identify the major proteins of both cell lines. Fig. 1 shows protein profiles of the studied cell lines. A visual inspection of protein patterns indicated that MCF-7 cell line shares a lot of similarities with the line MDA-MB-231. Significant differences in the electrophoretic profiles of both cell lines were observed at molecular masses of about 35, 44, 53, 100–120 and 210 kDa. Different as well as mutual strongest protein bands on the gels, marked in Fig. 1, were excised, in-gel digested with trypsin and gained peptide mixtures, originated from individual gel bands, were analyzed by MALDI-TOF MS. Additional MS/MS analysis of individual peptides and following database searching resulted in identification of the proteins summarized in Table 1.

MALDI-TOF MS analysis revealed 98 proteins across MCF-7 and MDA-MB-231 (Table 1). Out of which 60 were mutually differently expressed for MDA-MB-231 and MCF-7.

Identified proteins can be divided into several main groups: (1) actins; (2) actinins; (3) tubulins; (4) heat shock proteins; (5) annexins; (6) keratins and (7) others.

Actins (band 1, 16, 17), actinins (band 5, 6) and tubulins (band 13) represent major proteins observed in both studied cell lines. The most numerous group of heat shock proteins at complete scale of molecular weights is included in both cell lines (bands 4, 8, 9, 11). These proteins interact dynamically with various co-chaperones that modulate their substrate recognition, ATPase cycle and chaperone function. Furthermore, heat shock proteins, in general, are also involved in mode of steroid hormones action through

Table 1. Summary of identified proteins obtained by MS measurement

Band	MCF-7	MDA-MB-231	Protein	NCBInr number	Molecular mass (Da)
1	Y	Y	filamin A	gi|53791219	277332
	Y	Y	actin-binding protein homolog ABP-278	gi|3282771	278018
2	Y	-	fatty acid synthase	gi|915392	272919
	-	Y	myosin-9	gi|12667788	226392
	-	Y	talin	gi|6682361	269550
3	Y	Y	heterogeneous nuclear ribonucleoprotein U isoform b	gi|14141161	88924
	-	Y	karyopherin beta 3	gi|2102696	123512
4	Y	Y	nucleolin	gi|189306	76298
	Y	Y	ubiquitin activating enzyme E1	gi|35830	117715
	Y	-	nuclear corepressor KAP-1	gi|1699027	88479
	-	Y	100 kDa coactivator	gi|799177	99628
	-	Y	heat shock protein 70	gi|292160	78945
	-	Y	exportin-1	gi|4507943	123306
5	Y	Y	alpha actinin 4	gi|2804273	102204
	Y	Y	endoplasmin precursor	gi|4507677	92411
	Y	Y	glucosidase II	gi|2274968	106833
	Y	Y	splicing factor, proline- and glutamine-rich	gi|4826998	76102
	Y	-	keratin 1	gi|7331218	65978
	-	Y	alpha-actinin-1 isoform b	gi|4501891	102993
	-	Y	unnamed protein product (= alpha actinin 1)	gi|28334	103480
	-	Y	chain A, the crystal structure of human muscle alpha-actinin	gi|731187793	101834
	-	Y	KIAA0088 (= alpha glucosidase)	gi|577295	107158
6	Y	Y	elongation factor 2	gi|4503483	95277
	Y	Y	transitional endoplasmic reticulum ATPase	gi|6005942	89266
	-	Y	alpha-actinin	gi|178058	103229
7	Y	Y	heat shock protein HSP 90-beta isoform a	gi|20149594	83212
	Y	Y	HSP90AA1 protein	gi|83318444	68329
	Y	Y	calnexin precursor	gi|10716563	67526
	Y	Y	tumor necrosis factor type 1 receptor associated protein TRAP-1 – human	gi|1082886	75295
	-	Y	unnamed protein product (=heat shock protein HSP 90-alpha)	gi|32488	85020
	-	Y	probable Xaa-Pro aminopeptidase 3 isoform 1	gi|11559925	57034
	-	Y	importin subunit beta-1 isoform 1	gi|19923142	97108
8	Y	Y	GRP78 precursor, partial	gi|386758	72071
	Y	-	protein disulfide-isomerase A4 precursor	gi|4758304	72887
	Y	-	chain A, ATPase domain of human heat shock 70kDa protein 1	gi|6729803	41973
	Y	-	heat shock 70 kDa protein 8 isoform 1 variant	gi|62897129	71083
	-	Y	moesin	gi|4505257	67778
9	Y	Y	heat shock cognate 71 kDa protein isoform 1	gi|5729877	70854
	Y	Y	heat shock 70 kDa protein 9 (mortalin)	gi|12653415	73682
	Y	Y	heat shock 70 kDa protein 6	gi|34419635	70984
	Y	Y	HSP70-2	gi|4529892	69982
	Y	-	chain A, crystal structure of a heat shock 70 kDa protein 2	gi|395759492	42130
	-	Y	unnamed protein product	gi|194384180	54987
	-	Y	calelectrin	gi|179976	75857
	-	Y	X-ray repair cross-complementing protein 6 isoform 1	gi|4503841	69799
10	Y	Y	serum albumin	gi|28592	71316
	Y	-	unnamed protein product	gi|194388088	63885
	Y	-	similar to heat shock 70 kDa protein 8 isoform 2; heat shock cognate protein	gi|51095054	13379
	Y	-	growth regulated nuclear 68 protein	gi|226021	66881
	Y	-	transketolase	gi|37267	67751
	Y	-	dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit 1 precursor	gi|4506675	68527
	-	Y	lamin A protein, partial	gi|386856	57686

(continued)

Table 1. (continued)

11	Y	Y	60 kDa heat shock protein, mitochondrial	gi|31542947	61016
	Y	Y	pyruvate kinase	gi|35505	57841
	Y	Y	transformation upregulated nuclear protein	gi|460789	51040
	Y	-	keratin 1	See band 5	
	Y	-	thyroid hormone binding protein precursor	gi|339647	57069
12	Y	Y	calreticulin=calcium binding protein	gi|913148	3737
	Y	-	glucose-6-phosphate dehydrogenase	gi|26224790	54789
	Y	-	UDP-glucose 6-dehydrogenase isoform 1	gi|4507813	54989
	Y	-	peptidyl-prolyl cis-trans isomerase FKBP4	gi|4503729	51772
	-	Y	vimentin	gi|340219	53681
	-	Y	calreticulin precursor	gi|4757900	48112
13	Y	Y	tubulin alpha-1B chain	gi|34740335	50120
	Y	Y	tubulin beta-4B chain	gi|5174735	49799
	Y	Y	tubulin beta-5 chain	gi|7106439	49639
	Y	-	cytokeratin 8	gi|181573	53529
	Y	-	ATP synthase subunit beta, mitochondrial precursor	gi|32189394	56525
	-	Y	beta-tubulin	gi|338695	49727
	-	Y	alpha-tubulin	gi|37492	50810
14	Y	Y	alpha-enolase isoform 1	gi|4503571	47139
	Y	Y	elongation factor 1-gamma	gi|4503481	50087
	Y	-	cytokeratin 18 (424 AA)	gi|30311	47305
	Y	-	cytokeratin 8	See band 13	
	Y	-	protein kinase PKN beta	gi|6088096	99 421
15	Y	Y	phosphoglycerate kinase 1	gi|4505763	44586
	Y	-	glial fibrillary acidic protein isoform 1	gi|4503979	49850
	Y	-	keratin, type I cytoskeletal 19	gi|24234699	44079
	-	Y	eukaryotic initiation factor 4AII	gi|485388	46365
	-	Y	elongation factor Tu	gi|704416	49509
16	Y	Y	actin, cytoplasmic 1	gi|4501885	41710
	Y	Y	actin, alpha skeletal muscle	gi|4501881	42024
	Y	Y	beta-actin-like protein 2	gi|63055057	41976
	Y	-	40 kDa keratin protein, partial	gi|386803	44065
17	Y	Y	aldolase A	gi|28614	39307
	Y	-	actin prepeptide, partial	gi|178067	36783
	Y	-	cytokeratin 8 (279 AA)	gi|30313	30840
	Y	-	40S ribosomal protein S24 isoform C	gi|4506703	15413
	-	Y	40S ribosomal protein SA	gi|9845502	32833
	-	Y	laminin-binding protein,partial	gi|34234	32854
18	Y	Y	glyceraldehyde-3-phosphate dehydrogenase	gi|31645	36031
	Y	Y	chain R, twinning in crystals of human skeletal muscle D-glyceraldehyde-3-phosphate dehydrogenase	gi|230867	35853
	Y	-	myotubularin-related protein 14 isoform 2	gi|117938270	72158
	Y	-	40 kDa keratin protein, partial	gi|34039	44079
	Y	-	keratin 1	See band 5	
	-	Y	annexin A1	gi|4502101	38690
19	Y	Y	ribosomal protein L6	gi|36138	32841
	Y	-	fructose-1,6-bisphosphatase	gi|182311	36805
	-	Y	annexin A2	gi|18645167	38552
20	-	Y	chain A, the effect of metal binding on the structure of annexin	gi|809185	35783

Y, line consisting of the protein.

their cognate nuclear receptors (Beato and Klug 2000) and in the response to stress (Beere 2004). Within 32 proteins detected only in estrogen receptor-positive MCF-7 cells, fatty acid synthase expression and activity have been shown to be mediated by the G protein-coupled estrogen receptor in cancer cells and cancer-associated fibroblasts that strongly contribute to cancer progression (Santolla et al. 2012). Also, the relationships among estrogen receptor, progesterone receptor and glucose-6-phosphate dehydrogenase activity have been demonstrated in MCF-7 cells (Nerurkar et al. 1990). Within 30 proteins detected in estrogen receptor-negative MDA-MB-231 cells, moesin plays a marked role in the cancer cell invasiveness and pattern of metastasis characteristic of estrogen receptor-negative breast cancers (Carmeci et al. 1998). All-*trans* retinoic acid acting through nuclear retinoid receptors was found to increase the expression of all-*trans* retinoic acid receptor beta causing an inhibition of the 60% in cell migration and significantly decreases the expression of moesin and other migration-related proteins (Flamini et al. 2014).

Among proteins with regard to early cancer detection, of importance are predominantly heat shock protein HSP 90-beta isoform a (band 7), or heat shock 70 kDa protein 8 isoform 1 (band 8).

As expected, some well-known human breast cancer markers, such as annexins were also identified in this experiment. According to our results, annexin A1 and A2 (band 18 and 19) are selectively expressed in the invasive/metastatic MDA-MB-231 cells but not in the invasive/nonmetastatic MCF-7. Generally, annexins are proteins capable to reversibly bind to negatively charged phospholipids in a Ca^{2+}-mediated manner (Moss and Morgan 2004; Laohavisit and Davies 2009; Peng et al. 2014). Annexin A1 seems to act as a tumour suppressor in cells. Strong down-regulation of annexin A1 has been described at prostate, head, neck, and esophageal cancers, and on the other hand, up-regulation at hepatocarcinoma and pancreatic cancer (Ang et al. 2009). Increased expression of annexin A2 is also frequently observed in a broad spectrum of cancer cells (Wang and Lin 2014).

In contrast, only MCF-7 cells were found to be positive for cytokeratins and keratins (band 5, 11, 13, 14, 15, 16, 18). This finding is in a good agreement with results of Whelan et al. (2012) who described keratin 8 and 18 as other potential biomarkers in conjunction with keratin 19.

In the group marked as "others", we have found a lot of differently expressed proteins. The protein (band 12), which was identified from the spot about 50 kDa, represents an example of noticeable differences in the electrophoretic profiles of both cell lines. It corresponds to vimentin, specific protein for MDA-MB-231, which has been known to participate in a number of crucial functions, often related to organization of proteins that are involved in adhesion, migration, and cell signaling (Ivaska et al. 2007). Expression of this mesenchymal marker only in MDA-MB-231 cells and its non-detectability in the MCF-7 cells have also been described by other authors (Blick et al. 2008).

In conclusion, using basic proteomic analysis, we compared two different human breast cancer cell lines – MCF-7 and MDA-MB-231. Our attention was focused predominantly on major differences in their protein pattern, since, the differences in their phenotypes, especially from the protein composition point of view, have not been taken into account by many scientists (Mladkova et al. 2010). MALDI-TOF MS revealed almost one hundred proteins and 30 of them seem to be either up-regulated or specific to MDA-MB-231 in comparison with MCF-7 cells.

Thus, we presented the usefulness of this approach that can be utilized for basic comparisons of protein patterns of selected or unknown and new cell lines. Additionally, in this way, it is possible to specify a set of proteins recorded only in the particular lines, as we have shown in this work. Subsequently a decision about usage of the cells can be made based on their above mentioned proteomic similarity or dissimilarity.

Acknowledgements. This work was supported by APVV-0160-11, VEGA 2/0171/14, institutional support RVO:68081715 of the Institute of Analytical Chemistry of the CAS, v. v. i., SAV-15-01 and SAV-AVCR-15-01 grants.

References

Ang E. Z., Nguyen H. T., Sim H. L., Putti T. C., Lim L. H. (2009): Annexin-1 regulates growth arrest induced by high levels of estrogen in MCF-7 breast cancer cells. Mol. Cancer Res. **7,** 266–274 http://dx.doi.org/10.1158/1541-7786.MCR-08-0147

Beato M., Klug J. (2000): Steroid hormone receptors: an update. Hum. Reprod. Update **6,** 225–236 http://dx.doi.org/10.1093/humupd/6.3.225

Beere H. M. (2004): „The stress of dying": the role of heat shock proteins in the regulation of apoptosis. J. Cell Sci. **117,** 2641–2651 http://dx.doi.org/10.1242/jcs.01284

Bialesova L., Novotna A., Macejova D., Brtko J., Dvorak Z. (2015): Agonistic effect of selected isoflavones on arylhydrocarbon receptor in a novel AZ-AhR transgenic gene reporter human cell line. Gen. Physiol. Biophys. **34,** 331–334 http://dx.doi.org/10.4149/gpb_2015008

Blick T., Widodo E., Hugo H., Waltham M., Lenburg M. E., Neve R. M., Thompson E. W. (2008): Epithelial mesenchymal transition traits in human breast cancer cell lines. Clin. Exp. Metastasis **25,** 629–642 http://dx.doi.org/10.1007/s10585-008-9170-6

Cailleau R., Young R., Olive M., Reeves W. J. Jr. (1974): Breast tumor cell lines from pleural effusions. J. Natl. Cancer Inst. **53,** 661–674

Carmeci C., Thompson D. A., Kuang W. W., Lightdale N., Furthmayr H., Weigel R. J. (1998): Moesin expression is associated with the estrogen receptor-negative breast cancer phenotype. Surgery **124,** 211–217
http://dx.doi.org/10.1016/S0039-6060(98)70122-9

Farinha A. P., Irar S., de Oliveira E., Oliveira M. M., Pages M. (2011): Novel clues on abiotic stress tolerance emerge from embryo proteome analyses of rice varieties with contrasting stress adaptation. Proteomics **11,** 2389–2405
http://dx.doi.org/10.1002/pmic.201000570

Flamini M. I., Gauna G. V., Sottile M. L., Nadin B. S., Sanchez A. M., Vargas-Roig L. M. (2014): Retinoic acid reduces migration of human breast cancer cells: role of retinoic acid receptor beta. J. Cell Mol. Med. **18,** 1113–1123
http://dx.doi.org/10.1111/jcmm.12256

Ivaska J., Pallati H-M., Nevo J., Eriksson J. E. (2007): Novel functions of vimentin in cell adhesion, migration, and signaling. Exp. Cell Res. **313,** 2050–2062
http://dx.doi.org/10.1016/j.yexcr.2007.03.040

Keen J. C. (2011): Breast cancer cell line development and authentication. In: Breast Cancer - Focusing Tumor Microenvironment, Stem cells and Metastasis. (Eds. M. Gunduz and E. Gunduz), pp. 3–20, InTech Europe, Rijeka

Khan N. J. (2013): Cell lines: an in vitro model to study breast cancer. International Journal for Innovative Research in Science and Technology **2,** 7214–7219

Laohavisit A., Davies J. M. (2009): Multifunctional annexins. Plant Sci. **177,** 532–539
http://dx.doi.org/10.1016/j.plantsci.2009.09.008

Levenson A. S, Jordan V. C. (1997): MCF-7: the first hormone-responsive breast cancer cell line. Cancer Res. **57,** 3071–3078

Mladkova J., Sanda M., Matouskova E., Selicharova I. (2010): Phenotyping breast cancer cell lines EM-G3, HCC1937, MCF7 and MDA-MB-231 using 2-D electrophoresis and affinity chromatography for glutathione-binding proteins. BMC Cancer **10,** 449
http://dx.doi.org/10.1186/1471-2407-10-449

Moss S. E., Morgan R. O. (2004): The annexins. Genome Biol. **5,** 219
http://dx.doi.org/10.1186/gb-2004-5-4-219

Nerurkar V. R., Ishwad C. S., Seshadri R., Naik S. N., Lalitha V. S. (1990): Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities in normal canine mammary gland and in mammary tumours and their correlation with oestrogen receptors. J. Comp. Pathol. **102,** 191–195
http://dx.doi.org/10.1016/S0021-9975(08)80124-7

Peng B., Guo C., Guan H., Liu S., Sun M. Z. (2014): Annexin A5 as a potential marker in tumors. Clin. Chim. Acta **427,** 42–48
http://dx.doi.org/10.1016/j.cca.2013.09.048

Santolla M. F., Lappano R., De Marco P., Pupo M., Vivacqua A., Sisci D., Abonante S., Iacopetta D., Cappello A. R., Dolce V., Maggiolini M. (2012): G protein-coupled estrogen receptor mediates the up-regulation of fatty acid synthase induced by 17β-estradiol in cancer cells and cancer-associated fibroblasts. J. Biol. Chem. **287,** 43234–43245
http://dx.doi.org/10.1074/jbc.M112.417303

Soule H. D., Vasquez J., Long A., Albert S., Brennan M. (1973): Human cell line from a pleural effusion derived from a breast carcinoma J. Natl. Cancer Inst. **51,** 1409–1413

Wang C. Y., Lin C. F. (2014): Annexin A2: its molecular regulation and cellular expression in cancer development. Dis. Markers **2014,** 1–10
http://dx.doi.org/10.1155/2014/308976

Whelan S. A., He J. B., Lu M., Souda P., Saxton R. E., Faull K. F., Whitelegge J. P., Chang H. R. (2012): Mass Spectrometry (LC-MS/MS) identified proteomic biosignatures of breast cancer in proximal fluid. J. Proteome Res. **11,** 5034–5045
http://dx.doi.org/10.1021/pr300606e

Received: February 18, 2016
Final version accepted: February 26, 2016
First published online: May 13, 2016