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COMPARISON OF CELLULAR AND TISSUE TRANSCRIPTIONAL PROFILES IN CANINE MAMMARY TUMOR

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Tumor-derived cell lines are widely used as *in vitro* cancer models. Cell lines historically served as the primary experimental model systems for exploration of tumor cell biology and pharmacology. However, their ability to accurately reflect the phenotype and genotype of the parental histology remains questionable, given the prevalence of documented cell line-specific cytogenetic changes. Sometimes cell line studies are interpreted in the context of artifacts introduced by selection and establishment of cell lines *in vitro*. This complication has led to difficulties in the extrapolation of biology observed in cell lines to tumor biology *in vivo*. The aim of our study was to compare gene expression profiles in canine mammary tumor tissue and cell cultures derived from those tumors using cDNA microarrays. Tumors of two different origins were used; chondrosarcoma and adenocarcinoma and their primary cell cultures. It has been found that cell culture gene expression profiles closely resembled those of their corresponding *in vivo* tumor. In adenocarcinoma and chondrosarcoma only 6.0% and 2.7% of genes respectively, have shown significant difference in expression. In the most cases the difference concerned up-regulation of gene expression in cell lines, particularly genes involved in: protein metabolism and modification, signal transduction and nucleotide, nucleoside and nucleic acid metabolism. These experiments revealed that transcriptome of our primary cell culture corresponds to transcriptome of its parental tumor tissue and for this reason cell culture represents the reliable *in vitro* model for oncogenomic and pharmacogenomic studies.

Keywords: *canine mammary tumor, chondrosarcoma, adenocarcinoma, DNA microarray, tumor gene expression, cell line gene expression*

INTRODUCTION

Spontaneous mammary tumors are the most prevalent type of malignant neoplasm in the bitch and woman with the three times higher incidence in dog (1). About 50% of all mammary tumors are malignant (2). The etiology of breast cancer is very complex and not clearly understood. Genetic, hormonal, dietary, environmental and carcinogenic factors are known as the mediators of tumorigenesis (3). The role of estrogens, progestins and growth hormone in mammary gland development has been documented. Mainly affected are not spayed female dogs in the middle age. The early ovariectomy is thought to reduce the mammary cancer development risk to 0.05% (4). However, the high morbidity and

mortality rate, which is caused by not very effective treatment strategies (chemotherapy, immunotherapy, radiation) makes this problem still actual in both human and dogs. Cell lines derived from tumors and tissues have been historically instrumental for understanding of biology at the molecular level and are used in experimental research. There are general environmental differences between the cells growing *in vitro* and in heterogeneous tissue *in vivo*. The general differences in gene expression include an up-regulation of genes involved in proliferation and metabolism. Although cell lines differ from normal and tumor tissues, the low availability of tissue samples make cell lines the best model for future molecular cell biology research and drug development. New anticancer drugs are often developed with the help of cell lines, despite the fact that monolayer cultures are more sensitive than *in vivo* tumors.

The ability of cell lines to reflect precisely both phenotype and genotype of the parental histology still remains questionable. Sometimes cell line studies are interpreted in the context of artifacts introduced by selection and establishment of cell lines *in vitro*. Up to now, the comparison of gene expression profile between canine mammary tumor cell culture and parental tissue has not been performed.

The aim of our study was to compare gene expression profile between canine mammary tumors and cell lines derived from those tumors using cDNA microarrays. We examined tumors of two different origin: epithelial-adenocarcinoma and mesenchymal-chondrosarcoma and their primary cell lines.

MATERIALS AND METHODS

Media and reagents

Phosphate buffer saline, pH 7.4 (PBS), penicillin-streptomycin, fungizone and fetal bovine serum (FBS), were obtained from Gibco BRL (Gaithersburg, MD, USA). RPMI 1640 medium, BSA, TRIS buffered saline pH 8 and collagenase type 1 and all the other reagents were obtained from Sigma Chemicals (St. Louis, MO, USA). Antibodies to the immunohistochemical examinations: monoclonal mouse anti-human Ki-67, monoclonal mouse anti-human cytokeratin, clone MNF 116, monoclonal mouse anti-vimentin, clone Vim 3B4, monoclonal mouse anti - human muscle actin, clone HHF35, polyclonal rabbit anti-bovine S100 were obtained from DAKO Cytomation (Denmark) as well as En Visio ™ System and DAB. The monoclonal anti-human p63 antibody was obtained from Santa Cruz Biotechnology. DPX mounting medium derived from Gurr ®.

Glassware and plastics

Sterile conical flasks, sterile cell scrapers and sterile disposable pipettes were purchased from Nunc Inc. (Naperville, IL, USA).

Tumor sampling and cell isolation

Mammary adenocarcinoma was surgically removed during mastectomy in 12 years old mixed breed female. Chondrosarcoma was obtained during the mastectomy in 14 years old Cairn Terrier female. Tumors were divided into three pieces, one was fixed in 45 % formamid to perform histological assay, second piece was used to isolate and establish cell line. From the third part RNA was isolated for further cDNA microarray analysis.

Cell isolation

The tumor sample was collected into the medium RPMI 1640 containing flask immediately after the mastectomy. The tumor sample was then cut into the small pieces and cultured over the night in collagenase contained medium according to the Limon *et al.* (5) protocol (modification by Dr Eva Hellmen, Uppsala University, Sweden).

Cell culture

Cells were cultured in a medium RPMI-1640 enriched with 10% (v/v) heat-inactivated FBS, penicillin-streptomycin (50 iU/ml), fungizone (2.5 mg/ml), in an atmosphere of 5% CO_2/95% humidified air at 37°C and routinely subcultured every second day.

Immunohistochemistry and hematoxyline-eosin staining

The tumor samples (4 μm samples from paraffin blocks) and primary cell cultures were fixed on glass slides, stained with hematoxylin-eosin, and examined according to the WHO classification Midsorp (2). For further characteristic of cancer cells the tumor sections and corresponding cell lines were stained with antibodies against: Ki-67, cytokeratin, vimentin, muscle actin, S100 and p63. The standard procedure of staining was performed, according to the manufacturer recommendations. The pathological and immunohistochemical examination based on WHO classification with hematoxylin-eosin stain (tubules formation, mitotic index, degree of cell

differentiation) and antibodies reactions, showed that the first tumor was adenocarcinoma with chondro- and osteal- metaplasia, which originated from epithelium and the second one was chondrosarcoma tumor from mesenchymal origin.The findings from the same study (see Materials and Methods) made with cell cultures derived from those tumor tissues confirmed their origin (6).

RNA isolation and validation

Primary cell cultures isolated from the tumors were cultured until 90% confluence. The medium was next removed and replaced with PBS. Cells were scraped and total RNA from cell suspension was isolated using Total RNA kit (A&A Biotechnology, Poland) according to the manufacturer's protocol. Three replicates were performed (three different passages). RNA isolation from tumor tissue was performed with the RNAeasy Mini Kit (Qiagen) according to the manufacturer's protocol. Isolated RNA samples were dissolved in RNase-free water. Quantity of isolated RNA was measured using NanoDrop (NanoDrop Technologies, USA). The samples with adequate amount of RNA were treated with DNase I to eliminate DNA contamination. The samples were subsequently purified using RNeasy MiniElute Cleanup Kit (Qiagen, Germany). The quality of RNA was examined using Bioanalyzer (Agilent Technologies,USA).

Reverse transcription and cDNA labeling

Total RNA (10 µg) was reverse-transcribed using SuperScript Plus Indirect cDNA Labeling kit (Invitrogen, USA) according to the manufacturer's protocol. Single strand cDNA was labeled with Alexa 555 or Alexa 647 dyes (Invitrogen, USA). Efficiency of dyes incorporation was measured using NanoDrop (NanoDrop Technologies, USA).

Hybridization

Before hybridization, dog-specific cDNA microarray slides with 21 000 genes were prepared according to the recommended protocol. The cDNA microarrays were obtained from Dr Jan Mol from Utrecht University. Hybridization was performed using automatic hybridization station HybArray12 (PerkinElmer, USA). Slides were fixed in hybridization chambers and, after o-ring conditioning, samples of labeled cDNA were added. Hybridization of slides was performed using 18 h step-down hybridization protocol provided by the producer. After hybridization slides were washed automatically.

Signal detection, quantification and analysis

Acquisition and analysis of hybridization intensities were performed using microarray scanner ScanArray HT and ScanExpress software (PerkinElmer, USA). Different types of values were obtained for quantification of the dot intensity. Due to experimental variations in specific activity of cDNA target preparations or exposure time, that might alter the signal intensity, data from different hybridizations were automatically normalized (LOWESS method) (7) by ScanExpress software. Gene function was identified using the NCBI database and PANTHER pathway analysis software. Plots were done using ScanExpress (PerkinElmer, USA).and Excel Microsoft (Microsoft office, USA). For each comparison tissue-cell line three replications were performed. The differences between tissue and corresponding cell line transcripts were considered as significant when they repeated similarly in three replications with an average threshold of fold change. The average ratio of each spot on three slides was calculated using ScanExpress software. Genes that are 2-fold changed in three slides with value difference between the slides less than 5% were examined. This threshold is considered as a reliable border in gene expression difference between control and experimental sample .

RESULTS

Comparative analysis of transcriptomic profile of primary cell line derived from adenocarcinoma tumor versus adenocarcinoma tumor tissue

To compare the transcriptome of adenocarcinoma cell culture and tumor tissue, the cDNA microarray analysis was performed in 3 separate experiments and the genes with at least 2 time fold change were identified. This analysis identified 43 down-regulated and 229 up-regulated genes in the primary cell line versus adenocarcinoma tissue (*Table 1*). 4234 genes did not show any expression difference between examined samples. That makes only 6.0 % of genes, which differ in expression between cell culture and tumor tissue (*Fig. 1a*). Up- and down-regulated genes exhibited the fold change ranging between + 18.0 and - 6.7, respectively. Among the up-regulated genes in cell culture, the highest expression was observed in the case of acyl-Coenzyme A binding domain containing 5 (ACBD5, +18), apoptosis antagonizing transcription factor (AATF, +16.4), proteasome (prosome, macropain) activator subunit 2 (PSME2,+14), heat shock protein 90kDa alpha, class A member 1

Table 1. The list of genes up(+) and down (-) regulated in adenocarcinoma cell line in comparison to tumor tissue. Classification has been done according to their biological process.

No	Biological process	Transcript ID, gene name, gene symbol		Fold change
1	apoptosis	NM_012138, apoptosis antagonizing transcription factor	AATF	+16.4
		NM_178138, LIM homeobox 3	LHX3	+6.7
		NM_002775, HtrA serine peptidase 1	HTRA1	+5.6
		NM_005745, B-cell receptor-associated protein 31	BCAP31	+2.8
		NM_005427, tumor protein p73	TP73	-4
2	Cell adhesion	NM_033668, integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)	ITGB1	+4.4
		NM_004386, chondroitin sulfate proteoglycan 3 (neurocan)	CSPG3	+3.1
		NM_014914, centaurin, gamma 2	CENTG2	+3.1
		NM_021110, collagen, type XIV, alpha 1 (undulin)	COL14A1	+2.4
		NM_006157, NEL-like 1 (chicken)	NELL1	+2.1
		NM_015548, dystonin	DST	-3.4
		NM_004615, tetraspanin 7	TSPAN7	-3.4
3	Cell cycle	NM_012138, apoptosis antagonizing transcription factor	AATF	+16.4
		NM_016948, par-6 partitioning defective 6 homolog alpha	PARD6A	+11.5
		NM_000321, retinoblastoma 1 (including osteosarcoma	RB1	+8.5
		NM_003390, WEE1 homolog (S. pombe)	WEE1	+5.8
		NM_001239, cyclin H	CCNH	+5.8
		NM_002015, forkhead box O1A (rhabdomyosarcoma)	FOXO1A	+4
		NM_004153, origin recognition complex, subunit 1-like (yeast)	ORC1L	+3.1
		NM_002014, FK506 binding protein 4, 59kDa	FKBP4	+2.5
		NM_017575, Smg-6 homolog, nonsense mediated mRNA decay factor	SMG6	+2.2
		NM_002693, polymerase (DNA directed), gamma,	POLG	+2.1
		NM_005427, tumor protein p73	TP73	-4.1
4	Developemental processes	NM_001887, crystallin, beta B1	CYRBB1	+12.8
		NM_000496, crystallin, beta B2	CYRBB2	+12.8
		NM_016948, par-6 partitioning defective 6 homolog alpha	PARD6A	+11.5
		NM_004105, EGF-containing fibulin-like extracellular matrix protein 1	EFEMP1	+8.2
		NM_178138, LIM homeobox 3	LHX3	+6.9
		NM_000163, growth hormone receptor	GHR	+6.3
		NM_016407, chromosome 20 open reading frame 43	C20orf43	+5.9
		NM_001430, endothelial PAS domain protein 1,	EPAS1	+5.8
		NM_002775, HtrA serine peptidase 1,	HTRA1	+5.6
		NM_015120, Alstrom syndrome 1	ALMS1	+4.9
		NM_004441, EPH receptor B1,	EPHB1	+4.3
		NM_014930, zinc finger protein 510	ZNF510	+4
		NM_002015, forkhead box O1A (rhabdomyosarcoma),	FOXO1A	+4
		NM_004098, empty spiracles homolog 2	EMX2	+3.2
		NM_004386, chondroitin sulfate proteoglycan 3 (neurocan)	CSPG3	+3.2
		NM_003280, troponin C type 1 (slow),	TNNC1	+3.2
		NM_147127, Ellis van Creveld syndrome 2 (limbin)	EVC2	+3
		NM_002335, low density lipoprotein receptor-related protein 5	LRP5	+2.9
		NM_003247, thrombospondin 2	THBS2	+2.5
		NM_018194, hedgehog acyltransferase	HHAT	+2.8
		NM_021110, collagen, type XIV, alpha 1 (undulin),	COL14A1	+2.4
		NM_000321, retinoblastoma 1 (including osteosarcoma)	RB1	+2.4
		NM_078471, myosin XVIIIA	MYO18A	+2.4
		NM_006731, Fukuyama type congenital muscular dystrophy	FCMD	+2.3
		NM_006157, NEL-like 1 (chicken)	NELL1	+2.2
		NM_080749, neuralized homolog 2 (Drosophila)	NEURL2	-2
		NM_000430, platelet-activating factor acetylhydrolase, isoform Ib, alpha subunit 45kDa	PAFAH1B1	-3.5
		NM_012428, neuroplastin	NPTN	-3.5
		NM_182619, secretory protein LOC348174	unassigned	-4
		NM_173619, hypothetical protein MGC34761	unassigned	-4
		NM_005077, transducin-like enhancer of split 1 (E(sp1) homolog	TLE1	-4.1
		NM_018984, slingshot homolog 1 (Drosophila)	SSH1	-4.3
5	Nucleoside,nucleotide and nucleic acid metabolism	NM_012138, apoptosis antagonizing transcription factor,	AATF	+16.4
		NM_000321, retinoblastoma 1 (including osteosarcoma),	RB1	+8.5
		NM_178138, LIM homeobox 3,	LHX3	+6.9
		NM_005872, breast carcinoma amplified sequence 2,	BCAS2	+6.3
		NM_006414, ribonuclease P/MRP 38kDa subunit,	RPP38	+5.9
		NM_001430, endothelial PAS domain protein 1,	EPAS1	+5.8
		NM_014727, myeloid/lymphoid or mixed-lineage leukemia 4,	unassigned	+5.4
		NM_005648, transcription elongation factor B (SIII), polypeptide 1 (15kDa, elongin C),	TCEB1	+5.3
		NM_004735, leucine rich repeat (in FLII) interacting protein 1,	LRRFIP1	+5.2
		NM_206825, guanine nucleotide binding protein-like 3 (nucleolar),	GNL3	+4.5
		NM_012470, transportin 3,	TNPO3	+4.3
		NM_003418, CCHC-type zinc finger, nucleic acid binding protein,	CNBP	+4.2
		NM_014930, zinc finger protein 510,	ZNF510	+4
		NM_002015, forkhead box O1A (rhabdomyosarcoma),	FOXO1A	+4
		XM_942276, DEAH (Asp-Glu-Ala-His) box polypeptide 40,	DHX40	+4
		NM_175709, chromobox homolog 7,	CBX7	+3.8
		NM_003077, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 2,	SMARCD2	+3.4
		NM_022455, nuclear receptor binding SET domain protein 1,	NSD1	+3.3
		NM_004098, empty spiracles homolog 2 ,	EMX2	+3.2
		NM_004153, origin recognition complex, subunit 1-like (yeast),	ORC1L	+3.1
		NM_007144, polycomb group ring finger 2,	PCGF2	+2.8
		NM_005657, tumor protein p53 binding protein, 1,	TP53BP1	+2.3
		NM_002693, polymerase (DNA directed), gamma,	POLG	+2,1
		NM_003071, helicase-like transcription factor,	HLTF	+2.1
		NM_007062, PWP1 homolog ,	PWP1	+2
		NM_007279, U2 small nuclear RNA auxiliary factor 2	U2AF2	-3.5
		NM_005427, tumor protein p73	TP73	-4.1
		NM_005077, transducin-like enhancer of split 1 (E(sp1) homolog, Drosophila)	TLE1	-4.1
		NM_015523, REX2, RNA exonuclease 2 homolog (S. cerevisiae)	REXO2	-4.8
		NM_012323, v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)	MAFF	-5.3
		NM_014884, splicing factor, arginine/serine-rich 14	SFRS14	-5.5
6	oncogenesis	NM_000321, retinoblastoma 1 (including osteosarcoma),	RB1	+8.5
		NM_178138, LIM homeobox 3,	LHX3	+6.9
		NM_012308, F-box and leucine-rich repeat protein 11,	FBXL11	+4.9
		NM_005427, tumor protein p73	TP73	-4.1

7	Protein metabolizm and modification	NM_002818, proteasome (prosome, macropain) activator subunit 2 (PA28 beta),	PSME2	+14
		NM_005348, heat shock protein 90kDa alpha (cytosolic), class A member 1,	HSP90AA1	+13.5
		NM_183062, marapsin 2,	unassigned	+13
		NM_022450, rhomboid 5 homolog 1,	RHBDF1	+12
		NM_000985, ribosomal protein L17,	RPL17	+12
		NM_004314, ADP-ribosyltransferase 1,	ART1	+11
		NM_005869, serologically defined colon cancer antigen 10,	SDCCAG10	+10
		NM_015255, ubiquitin protein ligase E3 component n-recognin 2,	UBR2	+10
		NM_003812, ADAM metallopeptidase domain 23,	ADAM23	+6
		NM_080836, serine/threonine kinase 35,	STK35	+6
		NM_006656, sialidase 3 (membrane sialidase),	NEU3	+5.9
		NM_003390, WEE1 homolog (S. pombe),	WEE1	+5.8
		NM_006414, ribonuclease P/MRP 38kDa subunit,	RPP38	+5.8
		NM_002849, protein tyrosine phosphatase, receptor type, R,	PTPRR	+5.7
		NM_002827, protein tyrosine phosphatase, non-receptor type 1,	PTPN1	+5.6
		NM_002775, HtrA serine peptidase 1,	HTRA1	+5.6
		NM_139032, mitogen-activated protein kinase 7,	MAPK7	+5.3
		NM_006838, methionyl aminopeptidase 2,	METAP2	+5
		NM_012308, F-box and leucine-rich repeat protein 11,	FBXL11	+4.9
		NM_000986, ribosomal protein L24,	RPL24	+4.8
		NM_003333, ubiquitin A-52 residue ribosomal protein fusion product 1,	UBA52	+4.7
		NM_206825, guanine nucleotide binding protein-like 3 (nucleolar),	GNL3	+4.5
		NM_145214, tripartite motif-containing 11,	TRIM11	+4.3
		NM_004441, EPH receptor B1,	EPHB1	+4.3
		NM_031490, lon peptidase 2, peroxisomal,	LONP2	+4
		NM_005747, elastase 3A, pancreatic,	ELA3A	+3.9
		NM_013382, protein-O-mannosyltransferase 2,	POMT2	+3.5
		NM_006808, Sec61 beta subunit,	SEC61B	+3.4
		NM_006483, dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1B,	DYRK1B	+3.3
		NM_005830, mitochondrial ribosomal protein S31,	MRPS31	+3.2
		NM_002795, proteasome (prosome, macropain) subunit, beta type, 3,	PSMB3	+2.8
		NM_018194, hedgehog acyltransferase,	HHAT	+2.8
		NM_002014, FK506 binding protein 4, 59kDa,	FKBP4	+2.5
		NM_014752, signal peptidase complex subunit 2 homolog ,	SPCS2	+2.5
		NM_001015, ribosomal protein S11,	RPS11	+2.2
		NM_003751, eukaryotic translation initiation factor 3, subunit 9 eta, 116kDa,	EIF3S9	+2.1
		NM_015554, UDP-glucuronic acid epimerase	GLCE	-3
		NM_007106, ubiquitin-like 3	UBL3	-3
		NM_012288, translocation associated membrane protein 2	TRAM2	-3.6
		NM_145644, mitochondrial ribosomal protein	MRPL35	-3.7
		NM_199227, methionine aminopeptidase 1D	unassigned	-4
		NM_018984, slingshot homolog 1 (Drosophila)	SSH1	-4.3
		NM_017923, membrane-associated ring finger (C3HC4) 1	MARCH1	-5.3
		NM_000967, ribosomal protein L3	RPL3	-6.4
		NM_182640, mitochondrial ribosomal protein S9	MRPS9	-6.6
8	Signal transduction	NM_173598, kinase suppressor of ras 2,	KSR2	+8.5
		NM_004105, EGF-containing fibulin-like extracellular matrix protein 1,	EFEMP1	+8
		NM_000163, growth hormone receptor,	GHR	+6.3
		NM_003812, ADAM metallopeptidase domain 23,	ADAM23	+6
		NM_004688, N-myc (and STAT) interactor,	NMI	+5.7
		NM_002827, protein tyrosine phosphatase, non-receptor type 1,	PTPN1	+5.6
		NM_002775, HtrA serine peptidase 1,	HTRA1	+5.6
		NM_018084, KIAA1212,	KIAA1212	+5.4
		NM_014964, epsin 2,	EPN2	+5.3
		NM_139032, mitogen-activated protein kinase 7,	MAPK7	+5.2
		NM_001025201, chimerin (chimaerin) 1,	CHN1	+4.5
		NM_002071, guanine nucleotide binding protein (G protein), alpha activating activity polypeptide, olfactory type,	GNAL	+4.5
		NM_206825, guanine nucleotide binding protein-like 3 (nucleolar),	GNL3	+4.5
		NM_181532, ES cell expressed Ras,	ERAS	+4.4

(HSP90AA1,+13.6) and marapsin2 (+13.8) (*Table 1*). Conversely, the strongest down-regulated genes in cell culture were: mitochondrial ribosomal protein S9 (MRPS 9,-6.7), AATP-binding cassette, sub-family B (BCB1,-6.4), NLR family, purin domain containing 3 (NLRP3,-6.2), coatomer protein complex, subunit epsilon (COPE,-6.1) and transmembrane protein induced by TNFα (-5.5) (*Table1*).

Classification of differing genes according to their biological function revealed that they were engaged mainly in protein metabolism and modification (58 genes), signal transduction (44 genes) and nucleotide, nucleoside, and nucleic acid metabolism (40 genes) (*Fig. 2*).

Further analysis showed that 9 genes were expressed only in cell culture. They belonged to: phosphate metabolism (pyrophosphatase 1), sensory perception (EGF-containing fibulin-like extracellular matrix protein 1, forkhead box O1A, crystallin, beta B1) sulfur metabolism (thioredoxin-like 1, arylsulfatase G), coenzyme and prosthetic group metabolism (ferrochelatase) and carbohydrate metabolism (sialidase 3; forkhead box O1A (rhabdomyosarcoma) ; sorbin and SH3 domain containing 1) (*Fig. 2*).

Comparative analysis of transcriptomic profile of primary cell line derived from chondrosarcoma tumor versus chondrosarcoma tumor tissue

To compare the transcriptome of chondrosarcoma cell culture growing in optimal conditions and tumor tissue of its origin, we performed a cDNA microarray analysis in 3 separate experiments. In the course of the experiment we selected genes which expression differed similarly in three pairs of compared samples (with at least 2 times fold change).

We identified 31 down-regulated and 66 up-regulated genes in the primary cell line versus tumor tissue (*Table 2*), while 3446 did not show any

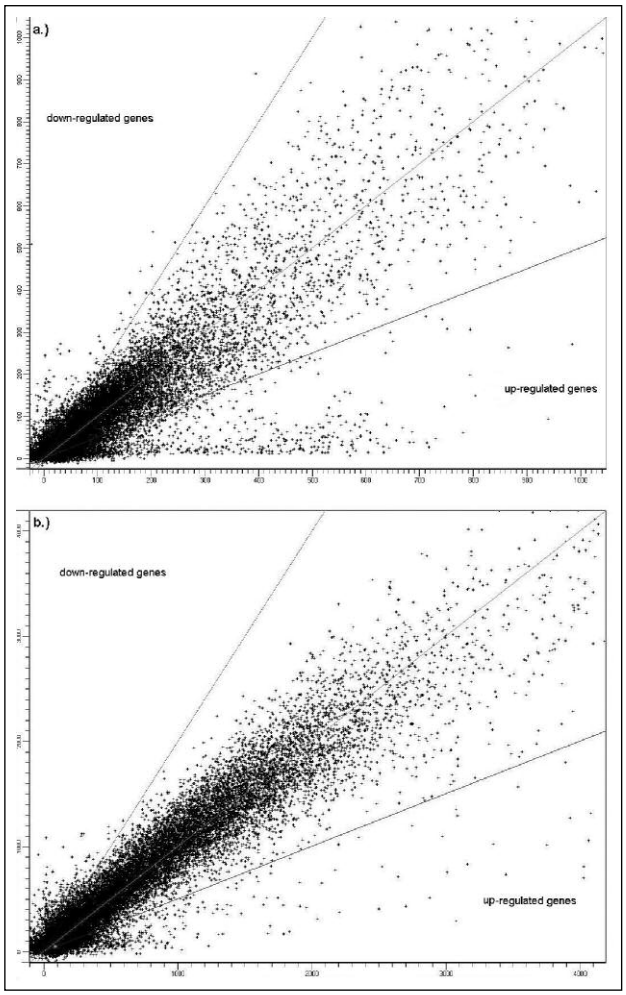

Fig. 1. Plots of adenocarcinoma (a) and chondrosarcoma (b) genes expression: x-axis shows the mean fluorescence of spots for tissue, while y-axis shows the mean fluorescence spots for cells. Lines above and below the center line denate the limits for 2-fold up- or down-regulation of gene expression in cell line in comparison to tumor tissue and points between these lines are considered unchanged between gene expression in tumor tissue and cell line.

difference in expression. It established 2,7% genes differing in expression between tumor tissue and its cell line (*Fig. 1 b*).Up- and down-regulated genes exhibited the fold change ranging between + 14 and -5, respectively.

Among the up-regulated genes in cell culture the highest expression was observed in five following genes (fold change between +14.0 and +5.9): leucine rich repeat containing 18 (LRRC 18), SEC22 vesicle trafficking protein homolog B (SEC 22 B), APAF1 interacting protein (APIP), adaptor-related protein complex 2, beta 1 subunit (AP2B1), MORN repeat containing 1 (MORN 1). Among the down-regulated genes in cell culture, the lowest expression (fold change between -5.0 and -3.0) was observed in the case of: phosphatase and actin regulator 3 (PHACTR3), adenomatosis polyposis coli (APC), suppressor of TY16 homolog (SUPT16H), cytochrome P450, family 2, subfamily C, (polypeptide 18 (CYP2C18), protein phosphatase 2, regulatory subunit B (B56), gamma isoform (PPP2R5C).

The genes, which exhibited the highest differences in expression between cell culture and tumor tissue were involved in biological processes such as: protein metabolism and modification (14 genes), signal transduction (14 genes) and nucleotide, nucleoside, and nucleic acid metabolism (12 genes) (*Fig. 3*). Among the up-regulated genes 10 were expressed only in cell culture. They were involved in: cell proliferation and differentiation (par-3 partitioning defective 3 homolog; peroxisome proliferator-activated receptor alpha; prohibitin 2), intracellular protein traffic (mitochondrial intermediate peptidase; adaptor-related protein complex 2, beta 1 subunit; peroxisomal biogenesis factor 7; SEC22 vesicle trafficking protein homolog B), sensory perception (lens intrinsic membrane protein 2, 19kDa) and protein targeting (intraflagellar transport 57 homolog; membrane protein, palmitoylated 7).

DISCUSSION

Cell lines are routinely used in experimental research as models for normal and pathological tissue and the results obtained from those experiments are extrapolated to a tissue and whole organism *in vivo*. In the present study, we have compared the transcriptomes of an *in vitro* model - canine mammary tumor-derived primary cell cultures to that of tumor tissue to determine the extent of differences in genes expression.

Primary cell culture derived from adenocarcinoma tumor expressed similary to tumor tissue up to 94% of all expressed genes, whereas chondrosarcoma cell culture showed even higher similarity to parental tissue gene expression: over 97% of all expressed genes. These values were obtained at the threshold of 2 fold change between gene expression in cell line and corresponding tumor tissue (*Fig.1*). These findings are similar to previously described studies performed on different types of tissue and their cell lines, where the differences in genes expression were not significant. B.A. Jessen *et al*. (8) and E.J Perkins *et al*. (9) described that similarity of liver genes expression in cell culture and tissue was 80% and 88%, respectively. A. Ertel *et al*. (10) showed as well similarity in the expression of the genes in cancer tissue and derived cell culture. Other studies (11) with tumor tissue and cell line of its origin confirmed that cell cultures can reflect *in vivo* tissue gene expression pattern and can be used for *in vitro* investigation of genomic alteration in cancers. However, the devised methods for

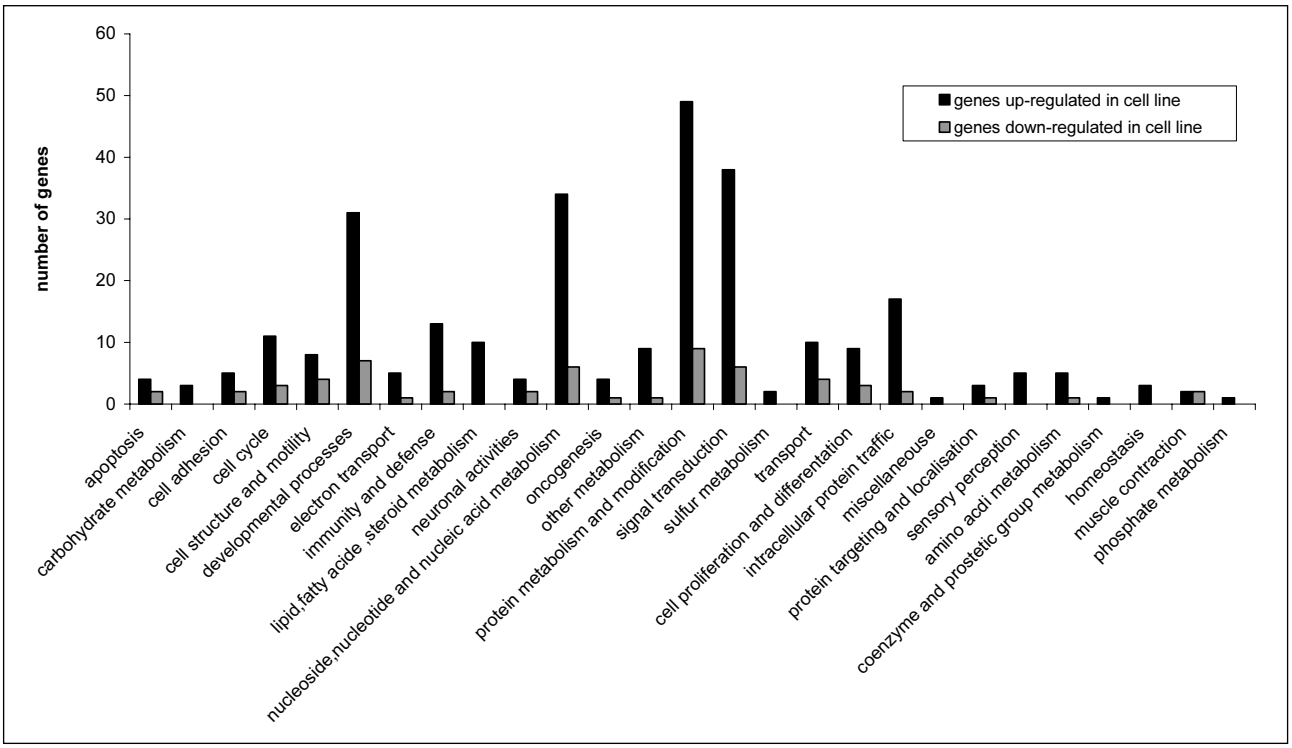

Fig. 2. Biological function of adenocarcinoma up- and down-regulated genes. Based on Panther database tools.

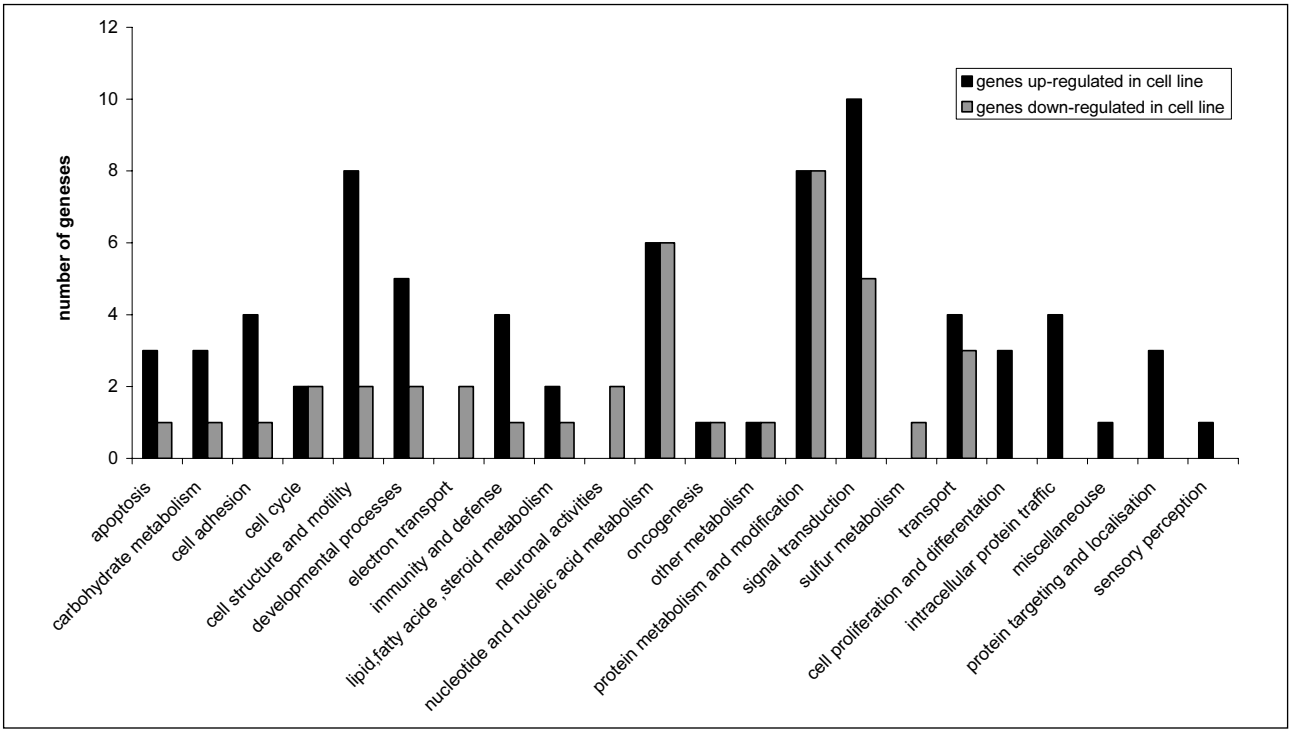

Fig. 3. Biological function of chondrosarcoma up- and down-regulated genes. Based on Panther database tools.

culturing cell lines do not reflect accurately the conditions present in the organism.

There are several factors which could be responsible for differences in gene expression *e.g.* changes in cells environment by their isolation and separation and the lack of the physiological signals from extracellular matrix, nervous and endocrine systems. The highest differences in gene expression concerned their up-regulation in cell lines, particularly in case of adenocarcinoma (*Table 1* and *2*). Among up-regulated genes, there were those responsible for some biological processes such as cell proliferation and differentiation, sensory perception, homeostasis, protein targeting and localisation,

Table 2. The list of genes up (+) and down (-) regulated in chondrosarcoma cell line in comparison to tumor tissue. Classification has been done according to their biological process.

No	Biological process	Transcript ID, gene name, gene symbol		Fold change
1	Apoptosis	NM_016068, fission 1 (mitochondrial outer membrane) homolog,	FIS1	+3.1
		NM_138278, BCL2/adenovirus E1B 19kD interacting protein like,	BNIPL	+3.2
		NM_018010, intraflagellar transport 57 homolog (Chlamydomonas)	IFT57	+3
2	Cell adhesion	NM_033130, sialic acid binding Ig-like lectin 10,	SIGLEC10	+2.5
		NM_019619, par-3 partitioning defective 3 homolog,	PARD3	+3.5
		NM_152888, collagen, type XXII, alpha 1,	COL22A1	+2
		NM_052884, sialic acid binding Ig-like lectin 11,	SIECLEC11	+2.5
3	Cell proliferation	NM_017493, OTU domain containing 4	OTUD4	+3.5
		NM_019619, par-3 partitioning defective 3 homolog	PARD3	+3.5
		NM_007273, prohibitin 2,	PHB2	+3.4
		NM_0010019288, peroxisome proliferator-activated receptor alpha	PPARA	+2.6
		NM_000038, adenomatosis polyposis coli	APC	-3.1
		NM_007192,suppressor of TY16 homolog	SUPT16H	-3.3
4	Developemental processes	NM_133265, angiomotin	AMOT	+5.4
		NM_006108, spondin 1, extracellular matrix protein	SPON1	+4.6
		NM_017493, OUT domain containing 4	OUT4	+3.5
		NM_001001928, peroxisome proliferator-activated receptor alpha	PPARA	+2.6
		NM_152991, embryonic ectoderma development	EED	-2
		NM_001145,angiogenin, ribonuclease, RNase A family	5ANG	-3
5	Intracellular protein trafic	NM_004892, SEC22 vesicle trafficking protein homolog B	SEC22B	+8.6
		NM_001282, adaptor-related protein complex 2, beta 1 subunit	AP2B1	+6.5
		NM_000288, peroxisomal biogenesis factor 7	PEX7	+3
		NM_005932, mitochondrial intermediate peptidase	MIPEP	+3
6	Nucleoside, nucleotide and nucleic acid metabolism	NM_007273, prohibitin 2	PHB2	+3.4
		NM_005997, vacuolar protein sorting 72 homolog	VPS72	+3.2
		NM_001693, ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B2	ATP6V1B2	+3.1
		NM_001001928, peroxisome proliferator-activated receptor alpha	PPARA	+2.6
		NM_005643, TAF11 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 28kDa	TAF11	+2.1
		NM_005574, LIM domain only 2 (rhombotin-like 1)	LMO2	+2
		NM_014828, KIAA0737	KIAA0737	-5
		NM_007192, suppressor of Ty 16 homolog	SUPT16H	-3.3
		NM_001145, angiogenin, ribonuclease, RNase A family, 5	ANG	-3
		NM_194430, ribonuclease, RNase A family, 4	RNASE4	-3
		NM_198232, ribonuclease, RNase A family, 1 (pancreatic)	RNASE1	-3
		NM_016316, REV1 homolog	REV1	-2.1
7	Protein metabolism and modification	NM_148976, proteasome (prosome, macropain) subunit, alpha type, 1	PSMA1	+3.7
		NM_022044, stromal cell-derived factor 2-like 1,	SDF2L1	+3.5
		NM_001025070, ribosomal protein S14,	RPS14	+3.4
		NM_016491, mitochondrial ribosomal protein L37	MRPL37	+3.2
		NM_005932, mitochondrial intermediate peptidase	MIPEP	+3
		NM_176863, proteasome (prosome, macropain) activator subunit 3 (PA28 gamma; Ki	PSME3	+2.6
		NM_001005, ribosomal protein S3	RPS3	+2.5
		NM_001281, tubulin folding cofactor B,	TBCB	+2.3
		NM_020117, leucyl-tRNA synthetase,	LARS	-2.1
		NM_004528, microsomal glutathione S-transferase 3	MGST3	-2.3
		NM_024105, asparagine-linked glycosylation 12 homolog	ALG12	-2.5
		NM_013382, protein-O-mannosyltransferase 2	POMT2	-2.8
		NM_003331, tyrosine kinase 2	TYK2	-3
		NM_003315, DnaJ (Hsp40) homolog, subfamily C, member 7	DNAJC7	-3.3
		NM_178587, protein phosphatase 2, regulatory subunit B (B56), gamma isoform	PPP2R5C	-5
8	Protein targeting and localization	NM_019619, par-3 partitioning defective 3 homolog	PARD3	+3.5
		NM_018010, intraflagellar transport 57 homolog (Chlamydomonas)	IFT57	+3
		NM_173496, membrane protein, palmitoylated 7 (MAGUK p55 subfamily member 7)	MPP7	+2.9
9	Signal transduction	NM_024848, MORN repeat containing 1	MORN1	+5.9
		NM_018653, G protein-coupled receptor, family C, group 5, member C	GPRC5C	+3.1
		NM_001001928, peroxisome proliferator-activated receptor alpha	PPARA	+2.6
		NM_018842, BAI1-associated protein 2-like 1	BAIAP2L1	+2.5
		NM_052884, sialic acid binding Ig-like lectin 11	SIGLEC11	+2.5
		NM_033130, sialic acid binding Ig-like lectin 10	SIGLEC10	+2.5
		NM_002567, phosphatidylethanolamine binding protein 1	PEBP1	+2.3
		NM_018460, Rho GTPase activating protein 15	ARHGAP15	2.2
		NM_004624, vasoactive intestinal peptide receptor 1	VIPR1	+2.1
		NM_172362, potassium voltage-gated channel, subfamily H (eag-related), member 1	KCNH1	-2.2
		NM_139136, potassium voltage-gated channel, Shaw-related subfamily, member 2	KCNC2	-2.3
		NM_003331, tyrosine kinase 2	TYK2	-3
		NM_000038, adenomatosis polyposis coli	APC	-3.1
		NM_178587 protein phosphatase 2, regulatory subunit B (B56), gamma isoform,	PPP2R5C	-5
10	transport	NM_001693, ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B2	ATP6V1B2	+3.1
		NM_000288, peroxisomal biogenesis factor 7	PEX7	+3
		NM_030657, lens intrinsic membrane protein 2, 19kDa	LIM2	+2.5
		NM_005669, receptor accessory protein 5	REEP5	+2.4
		NM_172362 potassium voltage-gated channel, subfamily H (eag-related), member 1	KCNH1	-2.2
		NM_139136, potassium voltage-gated channel, Shaw-related subfamily, member 2	KCNC2	-2.3
		NM_018849, ATP-binding cassette, sub-family B (MDR/TAP), member 4,	ABCB4	-2.5

intracellular protein transport, sulphur metabolism, phosphate metabolism and carbohydrate metabolism which were expressed only in cell lines (*Fig 2* and *3*). Up-regulation of some genes in cell culture can be explained by the action of growth stimulatory factors present in the incubation medium and the lack of suppressive effects of immune system occurring *in vivo*. In the previous studies the influence of some substances present in culture medium on the cell metabolism were described. R. Noding *et al*. (12) showed that polyunsaturated fatty acids as well as various pro- and antioxidants had an impact on growth of tumor cell cultures. It has been shown that cell line cultivated in RPMI 1640 was more sensitive for hydroperoxy docosahexaenoic acid (hp-DHA) then those growing in MEM-medium. This difference was explained by faster reduction of hp-DHA to the corresponding hydroxyl-DHA in MEM. Also addition of glutathione to the culture medium abolished the effect of hp-DHA. Some differences in gene expression may appear due to certain pathways being induced due to the trauma of cell isolation and adaptation to culture conditions (8). The fact that cells population in tumors is mixed, heterogeneous, existing of hypoxia conditions in growing tumor can have the alteration in genes expression.

Classification of differing genes according to their biological function revealed that in case of adenocarcinoma cell line the most of up-regulated genes were involved in protein metabolism and modification, signal transduction, nucleoside, nucleotide and nucleic acid metabolism and developmental processes (*Fig. 2*). In case of chondrosarcoma cell line up-regulation concerned mainly genes involved in: signal transduction, cell structure and motility and developmental processes. In this cell line genes involved in protein metabolism and modification and nucleotide and nucleic acid metabolism were equally up- and down-regulated (*Fig. 3*).

Classification of genes according to participation of their products in signalling pathways revealed that in adenocarcinoma cell culture up/down-regulation of expression concerned genes involved in Wnt pathway (LRP5, SMARCD2, HLTF, TLE1 and CTBP1), p53 pathway (RB1, TP73) and Alzheimer pathway (LRP5, MAPK7, CACNA1C); whereas in the case of chondrosarcoma cell culture genes were involved in FGF and EGF pathway (PEPB1, PPP2R2A, PPP2R5C). There are several studies showing that all these pathways are important in oncogenesis of mammary tumors (Brennan and Brown (13); Nusse and Varmus (14); Roelink *et al. (*15); Lee *et al.* (16); Tsukamoto *et al.* (17); Rao *et al.* (18,19); Liu *et al.* (20); Levine *et al.* (21); Bruns *et al.* (22); Roberts *et al.* (23); Fortin *et al.* (24)).

It has been shown that Wnt genes are overexpressed in both: canine mammary adenocarcinoma P114 cells, and in canine mammary gland tumors Rao *et al.* (19) .

In conclusion, primary cell lines express similar genes as canine mammary tumors and can developed the same pathways. Since transcriptomes of primary cell cultures correspond to transcriptomes of tumors tissues, cell cultures seem to be able to represent the reliable *in vitro* model for oncogenomic and pharmacogenomic studies.

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