Increased Expression of MCT4 and GLUT-1 in Early Events of Spheroid Formation of Adenocarcinomic Epithelial Cells A549

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Abstract: Monolayer cell cultures (2D) cannot adequately represent the growth and microenvironment conditions of three-dimensional (3D) tumors, resulting in false interpretation of molecular events and responses to chemotherapeutic interventions of solid tumors *in vivo*. Anaerobic conditions within an expanding spheroid, similar to *in vivo* conditions, cells are competing for nutrients and oxygen resulting in activated expression of metabolic enzymes in order to ensure an adequate supply of energy for cell proliferation. On the other hand cells growing in 2D cultures no such competition and no selective pressure exist. Therefore, *in vitro* multicellular tumor spheroid models provide a more reliable tool for drug screening and target identification. We established a screening strategy where small spheroids of A549 adenocarcinomic epithelial cells are studied. Induction of ITGA6 a marker in cellular polarity in 3D cultures could not be confirmed in our early formed spheroids. When these small colonies of cells are propagated under levitating conditions, nutrient and oxygen limitation is not relevant, therefore hypoxic induction of transcription machinery has not been initiated. We found that expression of genes coding for glycolytic enzymes PGK1, ALDOA1 and LDHA, some of the main indicators of cancer glycolytic pathway, was not changed significantly. In contrast, elevation of lactate transporter MCT4 and glucose transporter GLUT1 mRNA could be detected in 3D propagated cells. Interestingly, the other type of lactate transporter, MCT-1 expression was unaltered, therefore specific inhibition of MCT4 might prevent spheroid formation and growth. Our strategy for screening novel targets of early events of spheroid formation might identify proteins that may be promising pharmacological targets for cancer chemotherapy.

Keywords: Tumor spheroid, MCT4, GLUT1, ITGA6, Lactate transport.

INTRODUCTION

Monolayer cell cultures cannot adequately represent some aspects of the growth and microenvironment conditions of three-dimensional solid tumors, resulting in different responses to chemotherapeutic interventions. Therefore, *in vitro* models of multicellular 3D tumor spheroids provide an important tool for drug screening and target identification. On the other hand, experiments on animal models are time consuming, costly and not reasonable for high throughput screening studies. Therefore, *in vitro* models of multicellular tumor spheroids provide an important link between two-dimensional cell cultures and animal experiments [1-3].

The functional genomics studies using high throughput methods, such as microarrays, enable us to identify cancer-specific markers that can be utilized in diagnostics or in developing novel therapies [4-5]. During chemical genomics approaches, the integration of cell-based assays with activity/affinity-based approaches allows to interrogate the cells by analyzing phenotypic alterations, changes of transcript signature or detecting the differences in protein expression levels [6]. These approaches enable the investigator to obtain novel, therapeutically relevant targets as well as drug candidates in a target- or pathway-specific manner. However, when these studies are performed on 2D cell cultures gene, protein or phenotypic changes cannot adequately reflect the data obtained with solid tumors. By using DNA microarray technology Ernst *et al*. confirmed that gene expression changes in spheroid cultures closely resembled those detected in primary glioblastoma stem cell-like tumors obtained from patients, while genomic changes in monolayer cultures did not match well with the respective primary tumors [7].

Several studies have been published on applying cancer spheroids for screening and target identification [7-11], however, in these studies the subjects of investigations were the bigger spheroids that represent similar restricted conditions for the inner part of the spheroid cells to *in vivo* conditions. In those cells oxygen and nutrient deprivation result in anaerobic conditions, induced glyco-

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lytic enzymatic activities, elevated autophagy and necrosis [12-15].

In our study we intended to focus on the early events of colony formation in order to obtain more information on key genes that are responsible for metabolic switch and to better understand the early events of multicellular tumor formation.

MATHERIALS AND METHODS

Cell Line

The NSCLC cell line A549 (adenocarcinomic human alveolar basal epithelial cells) was purchased from the ATCC collection and maintained in suggested medium in a humidified atmosphere of 95% air and 5% CO_2.

2D Cell Culture

The A549 cancer cells were maintained in DMEM/F12 (DMEM, PAN^{TM} Biotech; F12 Nut mix, Gibco, Life Technologies) containing 4,5 g/L glucose and 10% fetal bovine serum (FBS, Gibco, Life Technologies), 1xGlutaMAX^{TM} (Gibco, Life Technologies) 1% PenStrep antibiotics (Penicillin G sodium salt, and Streptomycin sulfate salt, Sigma-Aldrich Co.). The cells were cultured in Tissue Culture T-75 flasks (Corning) reaching 70-80% confluence at standard atmosphere of 95% air and 5% CO_2. At about 70-80% confluence, cells were washed, harvested with collagenase and seeded into new T-75 flasks. Two independent experiments were conducted starting from different seeding cell numbers. Cells were harvested and analyzed after 2 and 4 days of incubation.

3D Cell Culture Using Bench-Top Incubator System

A549 cancer cells were cultured in specially designed LeviTubes^{TM} in the bench top bioreactor-incubator hybrid (BioLevitator^{TM}, Hamilton). During the cultivation, cells were proliferated in suspension culture without microcarriers with the following protocol: *Inoculation period:* Rotation Pause: 0 s, Rotation Period: 1 s, Agitation Pause: 20 min, Rotation Speed: 50 rpm, Agitation Period: 5 min, Duration: 5h. *Culture period:* Rotation Pause 0 s, Rotation Period: 1 s, Rotation Speed: 75 rpm, Duration: ∞.

LeviTubes^{TM} contained 40 ml media. Culture medium was changed once after 48 hour by removing half of the volume of original culture medium and replacing it with fresh media. Cells were collected by centrifugation at 3000 rpm at 4 ^{o}C for 10 min after 4 days rotation in the LeviTubes^{TM}.

Resazurin Viability Assay

The viability of A549 cells grown under 2D culture conditions, as well as the ones grown as spheroids in the Levitubes (Hamilton) was determined every day by the fluorescent resazurin (Sigma-Aldrich) assay. Resazurin reagent was dissolved in PBS (pH 7.4) at 0.15 mg/ml concentration, 0.22 µM filtered and aliquoted and stored at -20°C. After 2 hours incubation of cells with resazurin at 37°C, fluorescence was recorded (530 nm excitation / 580 nm emission) on a multimode microplate reader (Cytofluor4000, PerSeptive Biosytems). The relative growing kinetics was calculated by normalization to values obtained at day 0.

Nucleic Acid Isolation

RNA from 2D cell culture and spheroids was purified as described previously [16]. Briefly: columns and washing buffer were from Bioneer (Viral RNA extraction kit, Daejon, South Korea). Cells were washed with PBS, incubated in lysis buffer (RA1; Macherey-Nagel, Düren, Germany). The lysate was collected and mixed with 70% ethanol in RNase-free water (Bioneer). The mixture was transferred through columns. The columns were washed with 350 µl of 80% ethanol in diethylpyrocarbonate- (DEPC)-treated water. Then 95 µl DNase reaction mixture (Macherey Nagel, Düren, Germany) was loaded onto the columns and were incubated at room temperature for 15 min. After the DNase digestion, the columns were washed with 150 µl mixture of RA1 lysis buffer (Macherey-Nagel) and ethanol, 600 µl and 300 µl W2 washing buffer. The total RNA was eluted in 50 µl RNase free-water. One µl RNase inhibitor (Applied Biosystems, Foster City, CA, USA) was added to the samples. The quality and quantity of the isolated RNA were measured with NanoDrop1000 Version 3.8.1. (Thermo Scientific).

We started two and three replicates in monolayer cultures in the first and second experiments,

respectively. We started two replicates in 3D cultures both in the first and second experiments for nucleic acid isolation and subsequent gene expression analysis.

Gene Expression Analysis

Gene expression analysis was performed on cDNA converted from total RNA as described previously [17]. Briefly, reverse transcription from 3 µg total RNA in 30 µl was performed with the High Capacity cDNA Archive Kit according to the manufacturer's protocol (Applied Biosystems). cDNA was diluted to 80 µl. Gene expression was measured with gene-specific primers with SybrGreen protocol on a LightCycler® Nano Instrument (Roche). For cycling each 10 µl PCR reaction contained 20 ng cDNA, 5 µl FastStart Essential DNA Green Master (2x) (Roche), the corresponding primer set and the Setup Control. The primer sequences are listed in Table **1**. The PCR protocol was as follows: enzyme activation at 95 °C for 10 min, 50 cycles of denaturation at 95 °C for 15 sec, and annealing and extension at 60 °C for 30 sec. A total of 12 gene-specific assays were run on five independent samples from 2D condition and four independent samples from 3D conditions. Gene expression was normalized to the average values of beta actin (*ACTB*), glyceraldehyde-3-phosphate-dehydrogenase (*GAPDH*), and hypoxanthine phosphoribosyl transferase 1 (*HPRT1*) expression as endogenous controls and expressed relative to the average values of 2D cell culture controls (duplicates in the first experiment and triplicates in the second) by using the $2^{-\Delta\Delta Ct}$ method. Statistical analysis were performed using GraphPad Prism 6. Data were expressed as mean ± standard deviation (STD).

RESULTS

Cell Cultures

A549 cells were cultured using culture flask to obtain cells grown under 2D conditions. These cells served as controls for comparison of cells from 2D and spheroid cultures. To analyze whether different incubation times affect gene expression changes in 2D cultures 2 and 4 days incubation periods were applied in two independent experiments starting from the same seeding cell numbers. After reaching app. 70% confluency (Figure **1A**) cells were harvested for RNA preparation and subsequent gene expression study. Spheroid formation was performed in a special instrument, the Biolevitator™ system, where cells were levitated for 4 days. The cultivation of 2D and 3D cells were performed under the same conditions (cell culture media, glucose concentration, pH, pCO_2, pO_2). After 4 days levitation small spheroids consisting of 20-40 cells formed (Figure **1B**). To minimize

Table 1: Primers Used in This Study

Gene symbol	Product	Acc. No.	Forward primer	Reverse primer	Amplicon length
MMP9	matrix metallopeptidase 9	BC006093.1	gaaccaatctcaccgacagg	agggacagttgcttctggag	133
HIF1a	hypoxia inducible factor 1, alpha	AF304431.1	tcaagcagtaggaattggaaca	gtgatgtagtagctgcatgatcg	62
PGK1	phosphoglycerate kinase 1	BC113568.1	gcccagaagtggagaaagc	cacatgaaagcggaggttc	77
LDHA	lactate dehydrogenase A	NM_005566.3	gcagatttggcagagagtataatg	gacatcatcctttattccgtaaaga	93
MCT-1	solute carrier family 16, member 1	XM_005271150.1	gtgaccattgtggaatgctgt	catgtcattgagccgaccta	66
MCT-4	solute carrier family 16, member 3	BC112267.1	gctcacctcctccctgattt	tttgggcttcttcctaatgc	61
MMP2	matrix metalloproteinase 2	NM_004530.4	tgtgttctttgcagggaatg	ggtcagtggcttggggta	76
ENO1	enolase 1	M14328.1	ccttcatcaaggactacccagt	tcccccactacctggattc	109
ALDOA	aldolase A, fructose-bisphosphate	M11560.1	aagtacactccgagcggtca	ggcgtggttagagacgaaga	66
GLUT1	solute carrier family 2, member 1	BC118590.1	ccccatcccatggttcatc	tgaggtccagttggagaagc	91
ITGA6	integrin alpha 6	BC058095.1	tcagtattcaggagtagcttggtg	atcagaatcccggcaagaa	60
TRXR1	thioredoxin reductase 1	NM_003330.3	ggcgatatattggaggataagg	atagagcctctgagccagca	60
HPRT	hypoxanthine phosphoribosyltransf. 1	NM_000194.2	tgaccttgatttattttgcatacc	cgagcaagacgttcagtcct	102
ACTB	beta actin	BC002409.2	attggcaatgagcggttc	cgtggatgccacaggact	79
GAPDH	glyceraldehyde-3-phosphate dehydr.	BC025925.1	agccacatcgctgagaca	gcccaatacgaccaaatcc	66

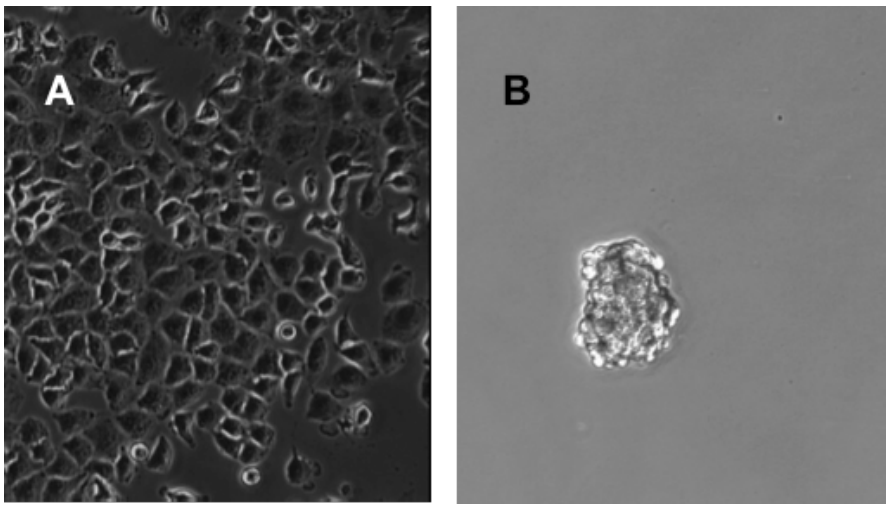

Figure 1: Two-dimensional (**A**) and spheroid (**B**) cell culture of A549 adenocarcinomic epithelial cells.

false positive and negative results spheroid cultivation was performed in two independent studies and 2 parallel samples were analyzed in each study. Spheroids were collected by centrifugation and after RNA purification and cDNA preparation gene expression analysis was conducted.

Functional difference between cells grown under 2D and 3D conditions was confirmed by analyzing their proliferation rate determined with the resazurin assay. While exponential proliferation could be detected in the 2D culture we found decreased proliferation of 3D cells in the course of the experiment (Figure **2**). At day 4, when the gene expression analysis was done three times less cells could be detected in the spheroids comparing to cells grown in 2D.

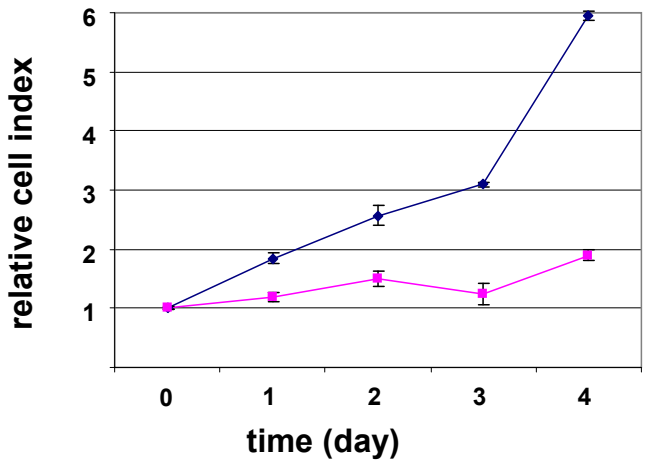

Figure 2: Proliferation rate of A549 cells grown under 2D (blue) and 3D (purple) conditions.

Gene Expression Analysis of 2D and Early 3D Cultures

Quantitative real-time PCR (QRT-PCR) was used to assess the expression of several genes involved in cancer metabolism (lactate dehydrogenase-A, LDHA; phosphoglycerate kinase 1, PGK1; lactate transporters MCT1 and MCT2; glucose transporter 1, GLUT1; enolase 1, ENO1 and aldolase, fructose-bisphosphate A, ALDOA), invasion (matrix metalloproteinase 2, MMP2 and matrix metalloproteinase 9, MMP9), cell surface adhesion (integrin subunit alpha 6, ITGA6) and oxidative stress (hypoxia inducible factor 1, alpha subunit, HIF1a and thioredoxin reductase-1, TRXR1).

Gene expression of these selected genes in spheroids was compared to that of 2D cells. Expression values were normalized to the average values of beta actin (*ACTB*), glyceraldehyde-3-phosphate-dehydrogenase (*GAPDH*), and hypoxanthine phosphoribosyl transferase 1 (*HPRT1*) expression as endogenous controls and expressed relative to the average of the 2D cell culture controls. Differential gene expression analysis revealed that among the analyzed genes, induction of lactate transporter MCT4 and glucose transporter GLUT1 genes was observed (Figure **3**). As a positive control, ITGA6, a cell polarization marker was used, as its significance in 3D cultures have already been proposed [18]. The most significant induction could be detected in case of MCT4, while interestingly the expression level of

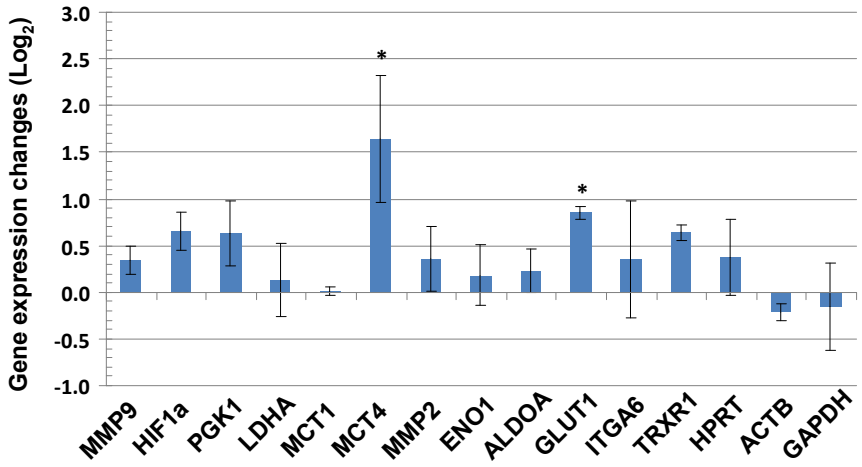

Figure 3: Differential gene expression analysis of two-dimensional (A) and spheroid (B) cell culture of A549 adenocarcinomic epithelial cells by using QRT-PCR technique. Gene expression changes are expressed as Log_2 values. *denotes for significance $p<0.05$ as calculated by T-test.

the other type of lactate transporter, MCT-1 was unaltered.

DISCUSSION

During tumor spheroid growth, oxygen and nutrient gradients develop, inducing specific genetic and metabolic changes in the proliferative and quiescent cellular layers. In our study we focused on the early events of colony formation in order to obtain more information on key genes that are responsible for metabolic switch and to better understand the early events of multicellular tumor formation.

Increased rates of glycolysis, including elevation of lactate dehydrogenase-A (LDHA) activity, are thought to be one of the hallmarks of tumor cells, which unlike normal cells, are able to produce lactate aerobically, a phenomenon known as the Warburg effect [20-21]. LDHA is a direct c-Myc-responsive gene that is involved in Myc-mediated cell transformation [22], and reduction of LDHA levels abrogates spheroid formation of c-Myc-transformed cells. It was hypothesized that the anaerobic conditions within an expanding spheroid colony of cells competing for nutrients and oxygen may select for cells with activated expression of metabolic enzymes such as LDHA to ensure an adequate supply of energy for cell proliferation [23]. Here we show that during the early phase of colony formation, under levitating conditions where nutrient and oxygen limitation is not relevant, LDHA expression was not altered.

Previously, it was shown that type VII collagen positively regulates the expression of ITGA6, a marker playing a role in cellular polarity in 3D spheroid keratinocyte cultures [18]. Other studies showed that silencing integrin α6 expression decreased the metastasis potential of hepatocellular carcinoma cells [24] and the loss of A6B4 expression inhibited colony formation of breast cancer cells in soft agar assays [25]. These results implicated a functional role for integrin α6 in anchorage-independent growth and in spheroid formation of cancer cells. In our study we found no change in ITGA6 expression under our experimental conditions indicating that cell polarization has not started yet in the early phase of 3D formation of lung adenocarcinomic epithelial cells.

Tumor cells produce large quantities of lactic acid via glycolysis. Monocarboxylate transporters (MCTs) are therefore commonly up-regulated in human malignancies to mediate lactic acid efflux. In our study, in the early phase of spheroid formation the induction of lactate transporter MCT4 and glucose transporter GLUT1 genes was observed. Monocarboxylate transporters (MCTs), also called the solute carrier family 16 (SLC16), especially MCT1 (SLC16A1) and MCT4 (SLC16A3) are crucial in regulating lactate entry or export through the plasma membrane and in decreasing high intracellular lactate levels resulting from glycolytic activity of cancer cells and/or cancer associated fibroblasts [26-28]. Of particular importance is the

upregulation of MCT4 expression in response to hypoxia, which is mediated by hypoxia inducible factor 1α (HIF1A) [29]. Malignant cells obtain their energy needs through increased glucose consumption, therefore tumor cells exhibit elevated levels of glucose uptake mediated by GLUT1, which is the key rate-limiting step in glucose utilization. GLUT1 expression is increased in various cancers [30-31]. This phenomenon has been used for diagnostic imaging in a wide variety of cancers with radio-labeled glucose analogs [32].

We hypothesize that MCT4 and GLUT1 induction precede the induction of other glycolytic genes and represents the first steps of reprogramming of tumor cells preparing them for 3D growth and altered metabolism.

Here we presented a novel screening platform where small spheroids levitating in oxygen and nutrient rich culture media were the subject of gene expression investigations. Global gene expression screening, as well as studies on proteome and metabolome of the small spheroids, might result in novel targets for cancer chemotherapy.

ACKNOWLEDGMENTS

This work was supported by the grant “Support of company R&D&I activities” which was submitted to the National Research, Development and Innovation Office (NKFIH), Hungary under the application number, GINOP-2.1.1-15-2015-00157.

REFERENCES

[1] Benien P, Swami A. 3D tumor models: history, advances and future perspectives. Future Oncol 2014; 10: 1311-1327.

[2] Mazzoleni G, Di Lorenzo D, Steimberg N, Modelling tissues in 3D: the next future of pharmacotoxicology and food research? Genes Nutr 2009; 4: 13-22.

[3] Lee GY, Kenny PA, Lee EH, Bissell MJ. Three-dimensional culture models of normal and malignant breast epithelial cells. Nat Methods 2007; 4: 359-365.

[4] Puskas LG, Juhasz F, Zarva A, Hackler L Jr, Farid NR. Gene profiling identifies genes specific for well-differentiated epithelial thyroid tumors. Cell Mol Biol (Noisy-le-grand) 2005; 51: 177-186.

[5] Puskás LG, Zvara A, Hackler L Jr, Micsik T, van Hummelen P. Production of bulk amounts of universal RNA for DNA microarrays. Biotechniques 2002; 33: 898-904.

[6] Darvas F, Dormán G, Krajcsi P, Puskás LG, Kovári Z, Lörincz Z, Urge L. Recent advances in chemical genomics. Curr Med Chem 2004; 11: 3119-3145.

[7] Ernst A, Hofmann S, Ahmadi R, Becker N, Korshunov A, Engel F, Hartmann C, Felsberg J, Sabel M, Peterziel H, Durchdewald M, Hess J, Barbus S, Campos B, Starzinski-Powitz A, Unterberg A, Reifenberger G, Lichter P, Herold-Mende C, Radlwimmer B. Genomic and expression profiling of glioblastoma stem cell-like spheroid cultures identifies novel tumor-relevant genes associated with survival. Clin Cancer Res 2009; 15: 6541-6550.

[8] LaBarbera DV, Reid BG, Yoo BH. The multicellular tumor spheroid model for high-throughput cancer drug discovery. Expert Opin Drug Discov 2012; 7: 819-830.

[9] Magdeldin T, López-Dávila V, Villemant C, Cameron G, Drake R, Cheema U, Loizidou M. The efficacy of cetuximab in a tissue-engineered three-dimensional *in vitro* model of colorectal cancer. J Tissue Eng 2014; 5: 2041731414544183.

[10] Hirschhaeuser F, Menne H, Dittfeld C, West J, Mueller-Klieser W, Kunz-Schughart LA. Multicellular tumor spheroids: an underestimated tool is catching up again. J Biotechnol 2010; 148: 3-15.

[11] Ravi M, Paramesh V, Kaviya SR, Anuradha E, Solomon FD. 3D cell culture systems: advantages and applications. J Cell Physiol 2015; 230:16-26.

[12] Amoêdo ND, Valencia JP, Rodrigues MF, Galina A, Rumjanek FD. How does the metabolism of tumour cells differ from that of normal cells. Biosci Rep 2013; 33 pii: e00080.

[13] Voss MJ, Niggemann B, Zänker KS, Entschladen F. Tumour reactions to hypoxia. Curr Mol Med 2010; 10: 381-386.

[14] Airley RE, Mobasheri A. Hypoxic regulation of glucose transport, anaerobic metabolism and angiogenesis in cancer: novel pathways and targets for anticancer therapeutics. Chemotherapy 2007; 53:233-256.

[15] Gonzalez CD, Alvarez S, Ropolo A, Rosenzvit C, Bagnes MF, Vaccaro MI. Autophagy, Warburg, and Warburg reverse effects in human cancer. Biomed Res Int 2014; 2014: 926729.

[16] Fabian G, Farago N, Feher LZ, Nagy LI, Kulin S, Kitajka K, Bito T, Tubak V, Katona RL, Tiszlavicz L, Puskas LG. High-Density Real-Time PCR-Based *in Vivo* Toxicogenomic Screen to Predict Organ-Specific Toxicity. Int J Mol Sci 2011; 12: 6116-6161.

[17] Catalá A, Zvara A, Puskás LG, Kitajka K. Melatonin-induced gene expression changes and its preventive effects on adriamycin-induced lipid

peroxidation in rat liver. J Pineal Res 2007; 42: 43-49.

[18] Dayal JH, Cole CL, Pourreyron C, Watt SA, Lim YZ, Salas-Alanis JC, Murrell DF, McGrath JA, Stieger B, Jahoda C, Leigh IM, South AP. (2014) Type VII collagen regulates expression of OATP1B3, promotes front-to-rear polarity and increases structural organisation in 3D spheroid cultures of RDEB tumour keratinocytes. J Cell Sci 2014;127: 740-751.

[19] Dhup S, Dadhich RK, Porporato PE, Sonveaux P. Multiple biological activities of lactic acid in cancer: influences on tumor growth, angiogenesis and metastasis. Curr Pharm Des 2012; 18: 1319-1330.

[20] Warburg O. On the origin of cancer cells. Science 1956; 123: 309–314.

[21] Racker E, Spector M. Warburg effect revisited: merger of biochemistry and molecular biology. Science 1981; 213: 1313.

[22] Pedersen PL. Tumor mitochondria and the bioenergetics of cancer cells. Prog. Exp. Tumor Res 1978; 22: 190–274.

[23] Shim H, Dolde C, Lewis BC, Wu CS, Dang G, Jungmann RA, Dalla-Favera R, Dang CV. c-Myc transactivation of LDH-A: implications for tumor metabolism and growth. Proc Natl Acad Sci U S A 1997; 94: 6658-6663.

[24] Lv G, Lv T, Qiao S, Li W, Gao W, Zhao X, Wang J. RNA interference targeting human integrin α6 suppresses the metastasis potential of hepatocellular carcinoma cells. Eur J Med Res. 2013; 18: 52.

[25] Lipscomb EA, Simpson KJ, Lyle SR, Ring JE, Dugan AS, Mercurio AM. The a6b4 integrin maintains the survival of human breast carcinoma cells *in vivo*. Cancer Res 2005; 65: 10970–10976.

[26] Halestrap AP, Wilson MC. The monocarboxylate transporter family - role and regulation. IUBMB Life. 2012; 64: 109–119.

[27] Martinez-Outschoorn U, Sotgia F, Lisanti MP. Tumor microenvironment and metabolic synergy in breast cancers: critical importance of mitochondrial fuels and function. Semin Oncol 2014; 41: 195-216.

[28] Pinheiro C, Longatto-Filho A, Azevedo-Silva J, Casal M, Schmitt FC, Baltazar F. Role of monocarboxylate transporters in human cancers: state of the art. J Bioenerg Biomembr 2012; 44: 127-139.

[29] Ullah MS, Davies AJ, Halestrap AP. The plasma membrane lactate transporter MCT4, but not MCT1, is up-regulated by hypoxia through a HIF-1alpha-dependent mechanism. J Biol Chem 2006; 281: 9030–9037.

[30] Younes M, Brown RW, Stephenson M, Gondo M, Cagle PT. Overexpression of Glut1 and Glut3 in stage I nonsmall cell lung carcinoma is associated with poor survival. Cancer 1997; 80: 1046–1051.

[31] Brown RS, Wahl RL. Overexpression of Glut-1 glucose transporter in human breast cancer. An Immunohistochemical Study. Cancer 1993; 72: 2979–2985.

[32] Adekola K, Rosen ST, Shanmugam M. Glucose transporters in cancer metabolism. Curr Opin Oncol 2012; 24: 650-654.

Received On: 21-December-2015 Accepted On: 11-Mar-2016 Published On: 25-Mar-2016