Tropical Journal of Pharmaceutical Research August 2019; 18 (8): 1589-1595
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v18i8.3

Original Research Article

Hypoxia-induced gene expression pattern in doxorubicin-resistant MCF7 cells

Hana M Hammad[1]*, Tuqa M Abu Thiab[1], Malek A Zihlif[2]
[1]Department of Biological Sciences, School of Science, [2]Department of Pharmacology, School of Medicine, The University of Jordan, Amman 11942, Jordan

For correspondence:* ***Email:* *hhammad@ju.edu.jo;* ***Tel:*** *+962 798507863*

Sent for review: 25 April 2019 *Revised accepted: 27 July 2019*

Abstract

Purpose: *To investigate hypoxia-induced gene expression pattern in doxorubicin-resistant human breast cancer cells (MCF7).*
Methods: *Human breast cancer cells (MCF7) were exposed to 60 episodes of 8 h hypoxia thrice a week for three months. Chemo-resistance to doxorubicin was assessed using 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) cell proliferation assay. Real-time quantitative polymerase chain reaction (qRT-PCR) assay was performed to assess gene expression pattern in doxorubicin-resistant cells on exposure to hypoxia.*
Results: *Hypoxia significantly increased the resistance of MCF7 cells to doxorubicin, with a maximum of 16.42-fold enhancement after 25 episodes of 8-h hypoxia, while the resistance thereafter significantly decreased with prolonged episodes of hypoxia ($p < 0.05$). Gene expression analysis revealed significant changes in 42 genes. The expressions of 10 of these genes were significantly upregulated, while those of 32 genes were significantly down-regulated ($p < 0.05$). Cytochrome P450 family 1, subfamily A, member1 (CYP1A1) was the most conspicuous upregulated gene (13.32-fold), while breast cancer gene 1 (BRCA1) was the most down-regulated (8.23-fold). Gene expression analysis after 60 episodes of 8-h hypoxia revealed the upregulation of CYP1A1 (5.77-fold). Similarly, 27 genes were significantly down-regulated, with BRCA2 as the most down-regulated gene (8.11-fold). Topoisomerase (DNA) II alpha (TOP2A) was the most down-regulated among genes involved in drug metabolism and resistance (6.37-fold), while cyclin-dependent kinase 2 (CDK2) was the most profoundly downregulated among genes involved in cell cycle regulation (3.56-fold).*
Conclusion: *These results indicate that development of resistance to doxorubicin by MCF7 cells after short-term hypoxia results from the upregulation of genes responsible for the metabolism of doxorubicin and for shifting the cells to alternative pathway driven principally by EGF and ESR2. The observed down-regulation is an adaptation of the MCF7 cells to survive under long-term hypoxia.*

Keywords: *Breast cancer cells, Doxorubicin, Chemoresistance, Hypoxia, Gene expression*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Cancer is a group of diseases that affect different parts of the human body. It starts with dysregulation of cell-cycle checkpoints, suppression of tumor-suppressor genes and activation of oncogenes. Factors related to the microenvironment play crucial roles in cancer initiation, progression, metastasis and radio-chemoresistance. An example of such factors is

hypoxia, which is a phenomenon shared by most tumors [1,2]. Abnormal vascularization and rapid proliferation of tumors results in fluctuation of oxygen supply throughout the growing mass [1,3].

Hypoxia enhances the ability of tumors to metastasize, and increases their aggressiveness and resistance to apoptosis [3]. It impacts significantly on radio-chemoresistance, resulting in poor prognosis in patients with severe hypoxic neoplastic tumors [2]. During radiotherapy, interaction between oxygen and radiation results in the production of oxygen free radicals which trigger DNA damage, thereby promoting apoptosis. On the other hand, hypoxic areas in large tumors are less susceptible to radiotherapy because of low levels of oxygen free radicals [4].

Several hypotheses have been formulated to explain the precise molecular mechanism(s) underlying chemoresistance of hypoxic tumors. These include somatic mutations, genetic instability, inadequate blood perfusion, and DNA over-replication [5]. The heterogeneous nature of cancers is responsible for observed differences in multi-drug resistance and varied expressions of genes responsible for such resistance [6]. The accompanying metabolic changes and cellular mechanisms triggered in response to hypoxia have not been fully elucidated. The present study investigated hypoxia-induced gene expression pattern in doxorubicin- resistant MCF7 cells.

EXPERIMENTAL

Materials

Human breast cancer cell line (MCF7) was obtained from American Type Culture Collection (ATCC, USA). Fetal bovine serum (FBS) and RPMI-1640 medium were purchased from Gibco (USA), while T75 tissue culture was obtained from SPL (Korea). Anaerogentm Compact was a product of Oxoid (USA). Assay kits for MTT were purchased from Promega (USA). Doxorubicin was obtained from Ebewe; Unterach (Austria). RNeasy® mini kit, RT2 first strand kit, RT2 SYBR® Green qPCR ready master mix, and RT2 profiler PCR array for human cancer drug resistance genes were products of Qiagen SABiosciences (USA).

Cell culture

The cells were first grown in T75 culture medium and allowed to attain 80 % confluency. They were thereafter cultured in RPMI-1640 medium supplemented with 10 % FBS, 1 % each of penicillin/streptomycin, L-glutamine (200 mM), and 1M HEPES buffer solution at 37 °C for 24 h in a humidified atmosphere of 5 % CO_2 and 95 % air. Cells in logarithmic growth phase were selected and used in this study.

Exposure to hypoxia

The cells were randomly assigned to two groups: normoxic (control) group and hypoxic group. Cells in hypoxic group were exposed to 60 episodes of 8-h hypoxia thrice a week for three months. The control group cells were maintained under normoxic conditions. An anaerobic atmosphere-generating system (Anaerogentm Compact) was used to generate hypoxia level less than 1 % [7].

Cell proliferation assay

Colorimetric MTT assay was used to determine the effect of hypoxia on doxorubicin resistance using non-radioactive cell proliferation MTT assay kit after every 5 episodes of hypoxia. The cells (1×10^4 cells/well) were seeded in 96-well plates and cultured in Dulbecco's modified Eagle medium (DMEM) for 24 h. Doxorubicin dilutions (starting with a concentration of 100 µM down to 15 dilutions) prepared in RPMI-1640 medium were added to the cells and incubated for 72 h. At the end of the third day, 20 µL of 5 g/L MTT solution was added to the wells, followed by incubation for another 4 h. The medium was finally replaced with 150 mL of 0.1 % dimethyl sulfoxide (DMSO) solution, agitated at 50 oscillations/min for 10 min to completely dissolve the formazan crystals formed, and absorbance of the samples was read in a microplate reader at 540 nm. The assay was performed in triplicate, and the half:maximum inhibitory concentration (IC_{50}) was determined. Hypoxia-induced resistance to doxorubicin was calculated as shown in Eq 1.

Resistance-fold = (IC_{50} of hypoxic MCF7 cells)/(IC_{50} of hypoxic MCF7 cells) …….. (1)

Quantitative real time polymerase chain reaction (qRT-PCR)

After every 5 episodes of hypoxia, the cells (5×10^6) were subjected to total RNA extraction using RNeasy® mini kit. The extracted total RNA (500 ng/µL) was converted to complementary DNA (cDNA) using RT2 first strand kit. The assay was performed in triplicate. The RT2 SYBR® Green qPCR ready master mix was used to prepare samples, and levels of expression of human breast cancer drug-resistant genes were investigated using RT2 profiler PCR array. Variation in the cDNA content was normalized

using β-actin. The PCR reaction mixture (20 µL) consisted of 6.4 µL of dH_2O, 1.6 µL of gene-specific primer (10 µM), 2 µL of synthesized cDNA and 10 µL of SYBR Premix Ex Taq™ II. The Ct value of U6 was taken as the internal parameter, and $2^{-\Delta\Delta Ct}$ was used to calculate the relative expression levels of the genes.

Statistical analysis

Numerical data are expressed as mean ± SD. Gene expression analysis was performed using GeneGlobe Data Analysis Center of SABiosciences. Genes with more than two-fold changes in expression levels were further analyzed and classified using Database for Annotation, Visualization and Integrated Discover (DAVID 6.8) Bioinformatics Resources [8].

Statistical analysis was performed using Graphpad Prism (6.0). Groups were compared using Student *t*-test. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Cellular resistance to doxorubicin

Table 1: Fold-change in resistance of MCF7 cells to doxorubicin induced by 8-h hypoxia episodes

No. of hypoxia episodes	Fold-change in resistance to doxorubicin
5	0.61 ± 0.017
10	1.34 ± 0.14
15	2.64 ± 0.15
20	7.91 ± 1.71
25	16.42 ± 3.84
30	2.30 ± 0.18
35	1.52 ± 0.07
40	2.07 ± 0.35
45	2.23 ± 0.25
50	1.46 ± 0.12
55	0.21 ± 0.03
60	0.57 ± 0.11

Hypoxia significantly increased the resistance of MCF7 cells to doxorubicin with a maximum of 16.42-fold increase after exposing cells to 25 episodes of 8-h hypoxia, but the resistance subsequently significantly decreased with prolonged episodes of hypoxia ($p < 0.05$; Table 1).

Changes in gene expression pattern after 25 episodes of hypoxia

Changes in gene expression were measured using PCR array for cells that showed maximum drug resistance. A standard 2-fold change in expression was used as an arbitrary cut-off. When cells exposed to 25 episodes of hypoxia were compared with their normoxic counterparts, expression analysis revealed significant changes in 42 genes. The expressions of 10 of these genes were significantly upregulated, while those of 32 genes were significantly down-regulated ($p < 0.05$). Cytochrome P450 family 1, sub-family A, member1 (CYP1A1) was the most conspicuous upregulated gene (13.32 fold), while BRCA1 was the most down-regulated gene (8.23 fold). These results are shown in Tables 2 and 3.

Changes in gene expression pattern after 60 episodes of hypoxia

Gene expression analysis after 60 episodes of 8-h hypoxia revealed the upregulation of CYP1A1 (5.77 folds) (Table 4). Similarly, 27 genes were significantly down-regulated, and BRCA2 was the most down-regulated gene (8.11 folds) (Table 5). Topoisomerase (DNA) II alpha (TOP2A) was the most down-regulated among genes involved in drug metabolism and resistance (6.37 folds) (Table 4), while CDK2 was the most profoundly down-regulated among genes involved in cell cycle regulation (3.56 folds). These results are shown in Table 6.

DISCUSSION

Hypoxia is one of the major factors responsible for resistance of tumor cells to therapies [2]. The adaptation of cancer cells to hypoxia involves an interplay of genetic and biochemical factors which promote cell survival, and contribute significantly to aggressiveness and resistance of the cells to therapy [1,2].

Table 2: Genes upregulated more than 2-fold in cells exposed to 25 hypoxic episodes

Gene	Description	Fold-change	Function
CYP1A1	Cytochrome P450 family 1 subfamily A member 1	13.32	Drug Metabolism
CYP2E1	Cytochrome P450 family 2 subfamily E member 1	4.27	Drug Metabolism
RELB	RELB proto-oncogene, NF-kB subunit	2.73	Transcription Factor
CYP1A2	Cytochrome P450 family 1 subfamily A member 2	2.4	
ARNT	Aryl hydrocarbon receptor nuclear translocator	2.28	Drug Metabolism
EGFR	Epidermal growth factor receptor/ (ERBB1, HER-1)	2.21	Growth factor receptors
GSTP1	Glutathione S-transferase pi 1	2.05	Drug metabolism
ATM	ATM serine/threonine kinase	2.03	DNA damage and repair

Table 3: Genes down-regulated more than 2-fold in cells exposed to 25 hypoxic episodes

Gene	Description	Fold-change	Function
BRCA1	DNA repair associated	-8.23	DNA Damage & Repair
DHFR	Dihydrofolate reductase	-7.11	Drug Metabolism
CDK2	Cyclin dependent kinase 2	-7.72	Cell Cycle
BRCA2	DNA repair associated	-6.3	DNA Damage & Repair
MSH2	MutS homolog 2	-6.12	DNA Damage and Repair
ERBB4	Erb-b2 receptor tyrosine kinase 4	-6.01	Growth Factor Receptors
TOP2A	Topoisomerase (DNA) II alpha	-5.73	Drug Resistance
BLMH	Bleomycin hydrolase	-5.57	Drug Metabolism
CCND1	Cyclin D1	-4.55	Cell Cycle
AR	Androgen receptor	-4.37	Hormone Receptors
CDK4	Cyclin dependent kinase 4	-4.35	Cell Cycle
PPARG	Peroxisome proliferator activated receptor gamma	-4.32	Hormone Receptors
CCNE1	cyclin E1	-4.32	Cell Cycle
BCL2	Apoptosis regulator	-4.31	Drug resistance
APC	WNT signaling pathway regulator	-3.98	DNA Damage & Repair
CYP2C8	Cytochrome P450 family 2 subfamily C member 8	-3.13	Drug Metabolism
MET	MET proto-oncogene, receptor tyrosine kinase	-3.13	Growth Factor Receptor

Table 4: Levels of expression of genes involved in drug metabolism and resistance after 60 hypoxic episodes

Gene	Description	Fold Change	Function
Upregulated genes			
CYP1A1	Cytochrome P450 family 1 subfamily A member 1	5.77	Drug metabolism
Downregulated genes			
TOP2A	Topoisomerase (DNA) II alpha	-6.37	Drug resistance
DHFR	Dihydrofolate reductase	-5.42	Drug metabolism
RB1	RB transcriptional corepressor 1	-3.35	Drug resistance
BLMH	Bleomycin hydrolase	-3.29	Drug metabolism
TOP1	Topoisomerase (DNA) I	-3.29	Drug resistance
SOD1	Superoxide dismutase 1, soluble	-2.87	Drug metabolism
TOP2B	Topoisomerase (DNA) II beta	-2.75	Drug resistance
NAT2	N-acetyltransferase 2	-2.47	Drug metabolism
BCL2	Apoptosis regulator	-2.34	Drug resistance
TPMT	Thiopurine S-methyltransferase	-2.32	Drug metabolism
UGCG	UDP-glucose ceramide glucosyltransferase	-2.04	Drug metabolism

Table 5: Genes involved in DNA damage and repair down-regulated after 60 hypoxic episodes

Gene	Description	Fold Change	Function
BRCA2	DNA repair associated	-8.11	DNA Damage & Repair
BRCA1	DNA repair associated	-6.68	DNA Damage & Repair
MSH2	MutS homolog 2	-4.62	DNA Damage and Repair
APC	WNT signaling pathway regulator	-2.42	DNA Damage & Repair
ATM	ATM serine/threonine kinase	-2.26	DNA damage and repair
ERCC3	ERCC excision repair 3, TFIIH core complex helicase subunit	-2.24	DNA Damage & Repair

Table 6: Genes involved in cell cycle regulation and transcription factors down-regulated after 60 hypoxic episodes

Gene	Description	Fold Change	Function
CDK2	Cyclin dependent kinase 2	-3.56	Cell Cycle
HIF1A	Hypoxia inducible factor 1 alpha subunit	-3.23	Transcription Factors
CDKN2D	Cyclin dependent kinase inhibitor 2D	-2.43	Cell Cycle
CCNE1	Cyclin E1	-2.17	Cell Cycle
CDK4	Cyclin dependent kinase 4	-2	Cell Cycle

Tumor cells employ hypoxia-inducible factor (HIF) family of transcription factors to adapt to hypoxic conditions, and the most studied member of this family is HIF-1, which has been linked to the aggressiveness and chemo-resistance of different tumors [2]. Other differentially regulated pathways have also been shown to contribute to hypoxia-induced drug resistance. However, these pathways have not been fully elucidated [5,6]. In this study, an attempt to find a suitable biomarker for cancer resistance during hypoxia led to gene expression profiling when the cells attained maximum resistance (after 25 episodes of hypoxia) and after 60 episodes of hypoxia. The results obtained showed that hypoxia plays an important role in the development of chemo-resistant phenotype in breast cancer cells. Distinct differences in gene expression were exhibited by cells exposed to 25 episodes of hypoxia, when compared with their normoxic counterparts.

The upregulated genes identified after 25 episodes of 8 h hypoxia are those involved in three important drug resistance mechanisms: drug inactivation, DNA damage repair, and cell cycle and cell death inhibition. In the drug inactivation group, four genes were upregulated: CYP1A1, CYP2E1, CYP1A2 and GSTP1. The upregulation of these genes may be responsible for the observed doxorubicin` resistance because the genes are responsible for metabolism of hydrocarbon compounds, including doxorubicin. It has been reported that these genes are overexpressed in doxorubicin-resistant cancer cells [9]. The upregulation of metabolizing enzymes may be due to the fact that these genes are activated via aryl hydrocarbon receptor nuclear translocator-1 (ARNT1) [10]. The ARNT1 gene is known to be upregulated in hypoxia. After the stabilization of hypoxia inducible factor 1 alpha (HIF-1α), ARNT1 dimerizes with HIF-1α, and on binding to the consensus hypoxia response element (HRE), it trans-activates downstream target genes involved in a variety of processes such as glycolysis, angiogenesis, proliferation, migration, and apoptosis [11].

The upregulation of three other genes: RELB Proto-Oncogene NF-KB Subunit (RELB), ATM serine/threonine kinase (ATM) and Retinoid X Receptor Beta (RXRB) ATM, may have contributed significantly to the observed doxorubicin resistance. Studies have shown that RELB, a member of NF-kB transcription factors family, is important in diverse biological processes such as inflammatory and immune responses, as well as cell survival and tumor progression [12,13]. Studies have shown that NF-κB prevents apoptosis via activation of target genes such as cellular inhibitors of apoptosis (cIAP1/2, XIAP) and cellular caspase8/FADD-like IL-1β-converting enzyme(FLICE)-inhibitory protein (c-FLIP) [12]. One of the key kinases involved in cellular response to DNA double-strand breaks is ATM. This gene exerts its effect by interacting with cyclin-dependent Kinase Inhibitor 1A (CDKN1A) and many other genes [14]. In this study, CDKN1A was significantly upregulated.

The retinoid X receptors (RXRs) are nuclear receptor transcription factors that bind retinoid. They are natural, as well as synthetic molecules structurally and/or functionally related to vitamin A, and regulate cell differentiation, proliferation, and survival [15]. A synergy between RXRB and activation of pancreatic stellate cells has been speculated to contribute significantly to resistance of MCF7 cells to doxorubicin.

Among the upregulated genes, epidermal growth factor receptor (EGFR) and estrogen receptor 2 (ESR2) drive cells through the cell cycle and promote their proliferation and chemo-resistance under hypoxic conditions. Changes in the level of expression of human epidermal receptors have been linked to the resistance of cancer cells to different anticancer therapies. Epidermal growth factor receptor (EGFR) promotes multidrug resistance via activation of the AKT/PKB and Ras/Raf/MEK/Erk pathways. Overexpression of EGFR has also been linked to the resistance of cancer cells to radiotherapy [17]. Estrogen receptor beta 2 (ERβ2) promotes cell proliferation and invasion [18]. The ERβ2 gene is highly expressed in aggressive castration-resistant prostate cancer cells, and has been shown to correlate with decreased overall survival [19]. In this study, ERβ2 was significantly upregulated in doxorubicin-resistant cells. This result is in agreement with those of previous studies [9].

The results of this study also showed that 35 genes were significantly down-regulated more than 2-fold after 25 episodes of 8-h hypoxia. These genes are most likely responsible for many cellular mechanisms. Interestingly, pathway analysis using DAVID ontology revealed that most of the genes are involved mainly in two important biological pathways: PI3K-Akt signaling pathway and cell cycle. It is likely that cancer cells in a hypoxic state try to avoid normal onco-activation of the cell cycle and normal cancer progression pathways regulated by PI3K/Akt signaling pathway. In this study, genes such as CCND1, CCNE2, and CDK2 that determine entry from G1 to S-phase of the cell cycle were significantly down-regulated. Similarly, genes

that regulate passage of cells through G2/M (CDK4 and TP53) were down-regulated. These results are in agreement with those of previous reports [20].

The down-regulation of PI3K-Akt signaling pathway can be explained on the premise that many mTOR upstream signaling pathways are controlled by both tumor suppressor tuberous sclerosis 1 and 2 (TSC1/2) complex and hypoxic regulation. The TSC1/2 complex negatively regulates the small GTPase ras-homolog-enriched-in-brain (RHEB). The activation of mTOR signaling pathway mediated by active RHEB leads to inhibition of TSC1/2 complex via phosphorylation [21]. Hypoxia is known to increase the AMP: ATP ratio, which leads to activation of AMP-activated protein kinase (AMPK) [22,23]. The AMPK phosphorylates TSC2, which activates TSC1/2 complex, while inhibiting RHEB. Inactive RHEB, in turn, cannot phosphorylate the mammalian target rapamycin (mTOR), thereby markedly reducing the effect of downstream signaling pathways mediated by BRCA1, BRCA2, cyclin D, c-myc P53 NF-kB and BCL2 [24]. The mTOR signaling also interacts with HIF-dependent hypoxic signaling pathway [22,23]. Studies have shown that HIF-1α is subjected to regulation by the PI3K/Akt/mTOR and PI3K/Akt/FRAP signaling pathways [20,25]. In addition, mTOR is an upstream mediator of HIF-1α activation [25]. The down-regulation of HIF-1α mRNA expression may potentially occur via mTOR signaling.

In the present study, many of the down-regulated genes such as BRCA1, BRCA2, ERCC2, MLH1 and MSH2 are involved in DNA damage repair pathways. It has been reported that hypoxic tumor microenvironment contributes to genetic instability through down-regulation of the expressions of DNA mismatch repair (MMR) genes (MLH1 and MSH2). This repression occurs via a dynamic shift from c-Myc/Max activating to repressive Mad1/Max and Mnt/Max complexes at the proximal promoters of both the MLH1 and MSH2 genes. Hypoxia-induced down-regulation of BRCA1 and BRCA2 gene expression has also been reported [27]. On the other hand, exposure of MCF7 cells to prolonged episodes of hypoxia resulted in the reversal of the observed resistance. This suggests that chemo-resistance decreases as the cells are exposed to greater shots of hypoxia. Indeed, most of the upregulated genes observed after 25 episodes of 8-h hypoxia showed no changes in expression pattern, except for CYP1A1 and ESR2 genes. Many of the down-regulated genes reported after 25 episodes of 8-h hypoxia were down-regulated after 60 episodes of 8 h hypoxia.

CONCLUSION

The results obtained in this study show that the development of resistance to doxorubicin by MCF7 cells after short-term hypoxia results from the upregulation of genes responsible for the metabolism of doxorubicin. These genes shift the cells to alternative pathways driven principally by EGF and ESR2. The observed down-regulation is an adaptation of the MCF7 cells to survive under long-term hypoxia.

DECLARATIONS

Acknowledgement

This work was supported with grant from The University of Jordan and Scientific Research Support Fund, Ministry of Higher Education and Scientific Research, Amman, Jordan (no. MPH/2/15/2015).

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

Open Access

REFERENCES

1. *Brahimi-Horn MC, Chiche J, Pouyssegur J. Hypoxia and cancer. J Mol Med. 2007; 85: 1301 - 1307.*
2. *Doktorova H, Hrabeta J, Khalil MA, Eckschlager T. Hypoxia-induced chemoresistance in cancer cells: The role of not only HIF-1. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2015; 159: 166 - 177.*
3. *Muz B, Puente P, Azab F, Azab AK. The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy. Hypoxia. 2015; 3: 83 - 92.*

4. Bindra RS, Crosby ME, Glazer PM. Regulation of DNA repair in hypoxic cancer cells. Cancer Metastasis Rev. 2007; 26: 249 - 260.
5. Kruh GD. Introduction to resistance to anticancer agents. Oncogene. 2003; 22: 7262 - 7264.
6. Gottesman MM. Mechanisms of cancer drug resistance. Annu Rev Med. 2002; 53: 615 - 627.
7. Hamdan FH and Zihlif M. Gene expression alterations in chronic hypoxic MCF7 breast cancer cell line. Genomics. 2014; 104: 477 - 481.
8. Huang W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc. 2009; 4: 44 - 57.
9. AbuHammad S, Zihlif M. Gene expression alterations in doxorubicin resistant MCF7 breast cancer cell line. Genomics. 2013; 101: 213 - 220.
10. Schofield CJ, Ratcliffe PJ. Oxygen sensing by HIF hydroxylases. Nat Rev Mol Cell Biol. 2004; 5: 343 - 354.
11. Liu ZJ, Semenza GL, Zhang HF. Hypoxia-inducible factor 1 and breast cancer metastasis. J Zhejiang Univ Sci B. 2015; 16: 32 - 43.
12. Karin M, Lin A. NF-kappaB at the crossroads of life and death. Nat Immunol. 2002; 3: 221 - 227.
13. Webster GA, Perkins ND. Transcriptional cross talk between NF-kappaB and p53. Mol Cell Biol. 1999; 19: 3485 - 3495.
14. Woods D, Turchi JJ. Chemotherapy induced DNA damage response: convergence of drugs and pathways. Cancer Biol Ther. 2013; 14: 379 - 389.
15. Polvani S, Tarocchi M, Tempesti S, Galli A. Nuclear receptors and pathogenesis of pancreatic cancer. World Journal of Gastroenterology. 2014; 20: 12062 - 12081.
16. Brun PJ, Wongsiriroj N, Blaner WS. Retinoids in the pancreas. Hepatobiliary Surgery and Nutrition. 2016; 5: 1-14.
17. Eccles SA. The epidermal growth factor receptor/Erb-B/HER family in normal and malignant breast biology. Int J Dev Biol. 2011; 55: 685 - 696.
18. Bialesova L, Xu L, Gustafsson J, Haldosen L, Zhao C, Dahlman-Wright K. Estrogen receptor beta2 induces proliferation and invasiveness of triple negative breast cancer cells: association with regulation of PHD3 and HIF-1alpha. Oncotarget 2017; 8: 76622 - 76633.
19. Dey P, Velazquez-Villegas LA, Faria M, Turner A, Jonsson P, Webb P, Williams C, Gustafsson J, Ström AM. Estrogen Receptor beta2 Induces Hypoxia Signature of Gene Expression by Stabilizing HIF-1alpha in Prostate Cancer. PLoS One. 2015; 10: e0128239.
20. Bertoli C, Skotheim JM, de Bruin RA. Control of cell cycle transcription during G1 and S phases. Nat Rev Mol Cell Biol. 2013; 14: 518 - 528.
21. DeYoung MP, Horak P, Sofer A, Sgroi D, Ellisen LW. Hypoxia regulates TSC1/2-mTOR signaling and tumor suppression through REDD1-mediated 14-3-3 shuttling. Genes Dev. 2008; 22: 239 - 251.
22. Arsham AM, Howell JJ, Simon MC. A novel hypoxia-inducible factor-independent hypoxic response regulating mammalian target of rapamycin and its targets. J Biol Chem. 2003; 278: 29655 - 29660.
23. Brugarolas J, Lei K, Hurley R, Manning BD, Reiling JH, Hafen E, Witters LA, Ellisen LW, Kaelin WG. Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. Genes Dev. 2004; 18: 2893 - 2904.
24. Giancotti FG. Deregulation of cell signaling in cancer. FEBS Lett. 2014; 588: 2558 - 2570.
25. Zhang Z, Yao L, Yang J, Wang Z, Du G. PI3K/Akt and HIF1 signaling pathway in hypoxia ischemia (Review). Mol Med Rep. 2018; 18: 3547 - 3554.
26. Mihaylova VT, Bindra RS, Yuan J, Campisi D, Narayanan L, Jensen R, Giordano F, Johnson R, Rockwell S, Glazer P. Decreased expression of the DNA mismatch repair gene Mlh1 under hypoxic stress in mammalian cells. Mol Cell Biol. 2003; 23: 3265 - 3273.
27. Fanale D, Bazan V, Caruso S, Castiglia M, Bronte G, Rolfo C, Cicero G, Russo A. Hypoxia and human genome stability: downregulation of BRCA2 expression in breast cancer cell lines. Biomed Res Int. 2013; 2013: 746858.