Disponible en ligne sur
SciVerse ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com

Annales d'Endocrinologie 74 (2013) 111–114

Annales d'Endocrinologie
Annals of Endocrinology

Journées Klotz 2013

Tumor hypoxia and metabolism – Towards novel anticancer approaches

L'hypoxie tumorale et le métabolisme – vers de nouvelles approches anticancéreuses

Johanna Chiche [a,*], Jean-Ehrland Ricci [a], Jacques Pouysségur [b,c]

[a] *Unité 1065, équipe « contrôle métabolique des morts cellulaires », Institut national de la santé et de la recherche médicale, centre méditerranéen de médecine moléculaire (C3M), 06204 Nice, France*

[b] *Institute for Research on Cancer and Aging of Nice, CNRS UMR 7284, University of Nice, Sophia-Antipolis, Centre A. Lacassagne, 06189 Nice, France*

[c] *Centre scientifique de Monaco, Centre A. Lacassagne, 06189 Nice, France*

Abstract

The transcription factor hypoxia-inducible factor-1 (HIF-1) facilitates the induction of enzymes necessary for regulation of biological processes required for cell survival and the acquisition of an aggressive and invasive phenotype, such as regulation of the intracellular pH (pHi), anaerobic glycolysis, angiogenesis, migration/invasion... In this presentation, we will highlight some of the HIF-1-induced gene products – carbonic anhydrases IX and XII (CAs) and monocarboxylate transporters (MCTs) – which regulate the pHi by controlling export of metabolically-generated acids (carbonic and lactic acids). We reported that targeting these pHi-regulated processes through inhibition of either HIF-1-induced CAIX/CAXII or HIF-1-induced MCT4, MCT1 or Basigin/EMMPRIN/CD147 chaperone of MCTs, severely restricts glycolysis-generated ATP levels and tumor growth. In addition, we demonstrated that the Myc/HIF-1-targeted glyceraldehyde-3-phosphate dehydrogenase (GAPDH) catalyzing a key step producing the NADH cofactor, activates the Akt pathway, thereby upregulating expression of the anti-apoptotic Bcl-xL. As a consequence, high expression of GAPDH contributes to tumor aggressiveness, in particular in the context *Myc*-driven B lymphomas. We propose that membrane-bound carbonic anhydrases (CAIX, CAXII), monocarboxylate transporters/chaperon Basigin (Myc-induced MCT1 and HIF-induced-MCT4) and GAPDH that are associated with exacerbated tumor metabolism, represent new potential targets for anticancer therapy.

Résumé

Le facteur de transcription induit par l'hypoxie (HIF-1) est responsable de l'induction de nombreux gènes impliqués dans l'adaptation et la survie des cellules tumorales soumises au stress hypoxique (diminution de la pression partielle en O_2) et dans l'acquisition d'un phénotype tumoral agressif. Parmi les gènes cibles de HIF-1 sont ceux qui participent par exemple, à l'angiogenèse, la régulation du pH intracellulaire, le métabolisme glycolytique, la migration/invasion et la résistance à l'apoptose. Au cours de cette présentation seront discutés les rôles de certaines protéines cibles de HIF-1, comme les anhydrases carboniques IX et XII et les transporteurs de monocarboxylate (MCT), dans la régulation du pH intracellulaire et le métabolisme glycolytique des cellules tumorales en condition d'hypoxie. Nous avons pu montrer que cibler la régulation du pH intracellulaire et/ou le métabolisme glycolytique via l'invalidation/inhibition de CAIX/CAXII ou de MCT1/MCT4 ou de la chaperone des MCT, la Basigin/EMMPRIN/CD147, compromet la production d'ATP et ralentit la croissance des cellules tumorales in vivo. De plus, nous avons récemment démontré que l'enzyme glycéraldéhyde-3-phosphate déshydrogénase (GAPDH), induite par HIF-1 et c-*Myc*, et qui catalyse une réaction glycolytique essentielle produisant le cofacteur nécessaire à la synthèse d'ATP (NADH), est capable de maintenir une activation basale d'Akt plus élevée que des cellules ne la surexprimant pas, conduisant ainsi à une surexpression de la protéine antiapoptotic BclxL. En conséquence, des niveaux élevés de GAPDH sont corrélés spécifiquement à une forte agressivité tumorale en particulier dans un modèle de souris développant des lymphomes B induits par c-*Myc*. L'identification de CAIX, CAXII, MCT1, MCT4, Basigin, GAPDH, comme associées à un métabolisme tumoral exacerbé, représente des opportunités thérapeutiques intéressantes afin de cibler l'avidité des cellules tumorales pour le glucose.

1. Hypoxic signaling and cancer metabolism

The pioneering investigation of Otto Warburg revealed that in contrast to normal cells, cancer cells have adapted by increasing glucose uptake and lactic acid production even in high oxygen tension, a phenomenon that has been widely described as the

* Corresponding author.
E-mail address: chiche@unice.fr (J. Chiche).

0003-4266/$ – see front matter © 2013 Published by Elsevier Masson SAS.
http://dx.doi.org/10.1016/j.ando.2013.02.004

"Warburg-effect" [1]. The Warburg-effect is the basis of tumor detection and their metastases by PET-SCAN imaging of the non-metabolized analog of glucose ^{18}FDG. Key aspects and signaling pathways involved in the Warburg-effect have now been elucidated. For example, genetic alterations such as *Myc* activation play a key role in this tumor « metabolic-shift » by inducing the expression of key glycolytic enzymes that results in extremely high rates of glycolysis and lactic acid production, a hallmark of aggressive cancers [2,3]. In addition, no matter what the oncogene mutation present, a key transcription factor responsible for the metabolic reprogramming of cancer cells from oxidative phosphorylation (OXPHOS) to glycolysis, is the Hypoxia-Inducible Factor-1 (HIF-1) [4,5]. Consequently, Myc and HIF-1 cooperate to increase tumorigenesis and cancer progression [6,7]. HIF-1 is a heterodimeric complex that is composed of two subunit, α and β. While mRNA of *hif-1α* and *hif-1β* are expressed in all tissues, oxygenated tissues constitutively express HIF-1β but lack HIF-1α proteins [8]. Indeed, HIF-1α expression is tightly regulated by oxygen levels. In oxygenated conditions (normoxia), the HIF-1α protein is continually degraded by the proteasome resulting in a half-life of less than ten minutes [9]. The rapid post-translational degradation of HIF-1α involves O_2 "sensors", the HIF prolyl-hydroxylases (PHD1, PHD2, and PHD3) that hydroxylate the HIF-1α protein to enable targeting of HIF-1α to the proteasome by the von Hippel Lindau (pVHL) ubiquitin E3-ligase complex [10,11]. As tumor cell develop, the supporting vasculature is often limiting, and not sufficient for tissues irrigation, leading to a deficient supply in oxygen (hypoxia). When a drop in pO_2 occurs, PHDs are inhibited which leads to an increased stability of HIF-1α resulting in the activation of the transcription factor HIF-1. Another O_2 "sensors" termed the Factor Inhibiting HIF (FIH) is an asparaginyl hydroxylase capable of inhibiting HIF-1 transcriptional activity of the C-terminal transactivation domain in the presence of O_2 [12]. In acute hypoxia, FIH is inhibited leading to full activity of HIF-1. Once stabilized, HIF-1α translocates to the nucleus where it interacts with HIF-1β and cofactors to bind specific DNA sequences known as hypoxia-responsive elements (HREs) [13]. HIF-1 activation is capable of modifying 1-2% of the genome [5] to adpat to a decrease in pO_2 and nutrients availability. Among HIF-1-induced genes are those involved in autophagy, decreased respiration, increased glycolysis, pH regulation, erythropoiesis, vasoldilatation, angiogenesis, migration/invasion [14]. Consequently, nuclear detection of HIF-1α by immunohistochemistry or the expression of the transcriptional "hypoxia-signature" corresponds with a poor prognosis as has been verified in a number of solid tumors [15,16]. Expression of HIF-1α and activity of HIF-1 is not exclusively correlated with a decrease in pO_2. Although the O_2-dependent hydroxylation, ubiquitylation, and degradation of the α subunit provide the primary means of regulating HIF-1 activity, other HIF-1 modulators have been described in recent years, complicating the picture. For example, activation of NFκB is shown to increase HIF-1α and HIF-1β levels by increasing their transcription [17,18]. Activation of PI3 K/Akt pathway (*via* deletion of PTEN or activating mutations of PI3 K) is also capable of increasing HIF-1α expression [19] by increasing its translation via the mTOR pathway [20]. In renal cancer, mutations in *vhl* are responsible for a constitutive expression of HIF-1α in normoxia. All of these data combine to implicate HIF-1 as a major player in the tumor "metabolic-shift" that results in extremely high rates of glycolysis and lactic acid production. Thus, the acidic tumor environment provides an appealing target for therapeutic applications.

2. Glucose metabolism, hypoxia and pHi regulation, offer novel anticancers strategies

Hypoxic cells have developed scenarios to maximally export intracellular acids such as lactic acids (H^+, lactate$^-$and carbonic acids (H^+, HCO_3^-). Indeed, to maintain cell survival in hypoxic conditions, tumor cells have to counteract the intracellular production of these acids. At the heart of this regulatory system is HIF-1 which contribution is summarized in the figure. There are actually number of research programs trying to poison tumor cells by their own production of acids to collapse ATP production [21]. Here, we demonstrate that novel markers of hypoxia and metabolism represent important targets for novel anticancer strategies.

Besides the constitutively expressed and widely characterized pH_i-regulating systems as the Na^+/H^+ exchangers NHE(s) and ATP-dependent H^+-pumps V-ATPase, we focused our interest in two hypoxic markers, the HIF-1-induced membrane-bound carbonic anhydrases IX and XII (CAIX and CAXII) that catalyze the hydration of CO_2 into HCO_3^- and H^+ (Fig. 1) [22,23]. Considering together the important influence of pH_i regulation on glycolytic metabolism, cell divison [24] and the buffer properties of HCO_3^-, we hypothesized that hypoxic tumor cells expressing these enzymes, in particularly CAIX, would acidify the extracellular media and would recapture HCO_3^- ions to maintain pHi values permissive with glycolysis, ATP production, cell survival, proliferation, migration [22] in hostile extracellular acidic environments (Fig. 1). Using overexpression of these enzymes, or specific targeting of CAIX and/or CAXII (via inducible and constitutive shRNA) we validated our predictions and demonstrated that both HIF-1-induced CAIX and CAXII are robust pHi-regulating system able to confer an in vivo growth advantage for tumors. As a consequence we established for the first time that the membrane-bound extracellular facing CAIX and CAXII can be considered as anticancer targets [23]. To date, only a few small molecules have been characterized in CA-relevant cell and animal model systems. From the recent development of a new class of carbohydrate-based sulfonamide CA inhibitors, we identified two CAIX and CAXII inhibitors that block CAIX-induced survival of fibroblasts and colon carcinoma cells in acidic environments within a range of low nanomolar values and hold potential for development as in vivo cancer cell selective strategy [25]. In PTEN-deleted lymphoma cells expressing high levels of CAXII, these two inhibitors demonstrated a strong reduction in cell survival. More recently, we also established that intracellular acidosis sensitizes cells to irradiation-induced cell death and that expression of CAIX protects cells against ionizing radiation [26]. Finally, our in vivo results highlight the combinatory use of

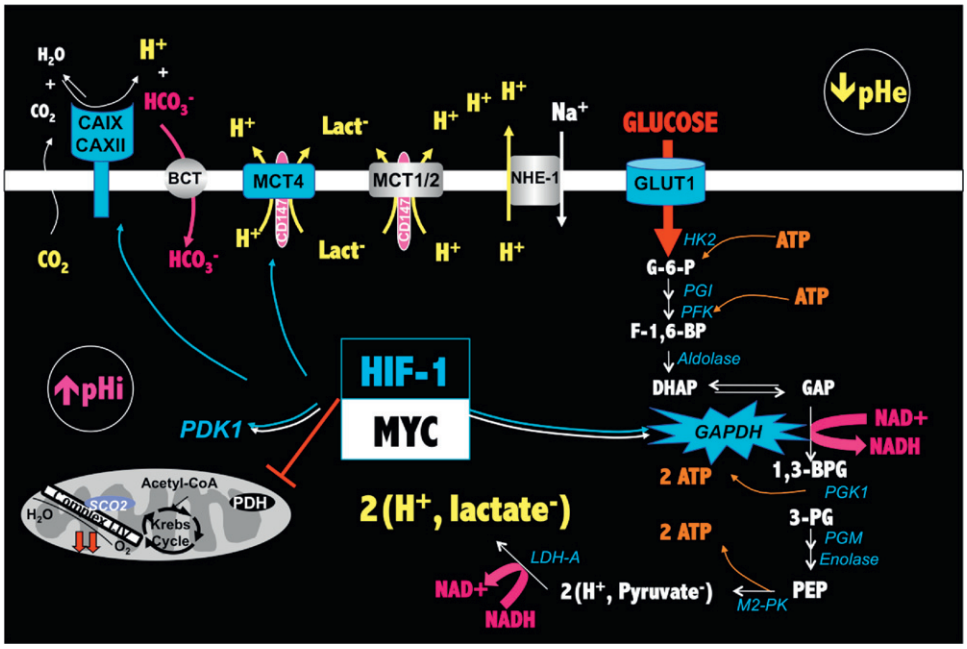

Fig. 1. Up-regulation of glycolysis is the hallmark of invasive cancers. The oncogene Myc and the Hypoxia-inducible factor-1 (HIF-1) stimulate glycolysis by activating the expression of the glucose transporters GLUT1,-4 and the key glycolytic enzymes hexokinase 2 (HK2), glyceraldheyde-3-phosphate dehydrogenase (GAPDH), lactate dehydrogenase (LDH-A), and pyruvate dehydrogenase kinase 1 (PDK1), an inhibitor of pyruvate dehydrogenase (PDH) that inhibits mitochondrial uptake of pyruvate. Consequently puruvate is redirected to be converted into lactic acid (H^+, $Lactate^-$) by the LDH-A. Despite the huge production of lactic acid, tumor cells and in particular hypoxic tumor cells maintain an intracellular pH (pHi) compatible with ATP production, cell viability and growth by inducing HIF-1 targeted genes as the membrane-associated carbonic anhydrases IX and XII (CAIX and CAXII) and the MonoCarboxylate Transporter 4 (MCT4), in addition to the constitutively expressed pHi-regulating systems Na^+/H^+ exchanger NHE-1, HCO_3^- transporters (BCT), MCT1, among others. Extrusion of acids leads to a decrease in the extracellular pH (pHe).

radiotherapy with targeting of CAIX and CAXII as an anticancer strategy [26].

Since lactic acid is the end product of glycolyisis, we also proposed that inhibiting its export would be a radical approach to inhibit glycolysis and ATP production via cellular acidification and lactic acid accumulation. As a consequence, we focused on lactic acid transporters also named MonoCarboylate Transporters (MCTs) and their ancillary protein CD147/Basigin required for MCT(s) functionality at the cell surface. Our goal was also to determine the contribution of each isoforms expressed in tumor cells, the ubiquitously expressed MCT1 and MCT2 and the HIF-1-induced MCT4 overexpressed in hypoxia and in highly glycolytic tissues [27]. Using a specific inhibitor of MCT1/2 (from Astranzeneca) we demonstrated that inhibition of MCT1/2 stopped in vivo proliferation of tumors lacking MCT4 (50% of melanomas lack MCT4), while re-expression of MCT4 in these cells conferred resistance to MCT1/2 inhibition and reestablished tumorigenicty [28]. Highly glycolytic cells lacking MCT4 and displaying low tumorigenicity (20% of the mice develop tumors in xenograft) were selected for resistance to MCT1/2 inhibitor. Resistant cells restored their capacity to generate tumor in all animals injected, due a reactivation of *mct4*, highlighting that the hypoxia-inducible MCT4 drives tumorigenicity [28]. Non-invasive quantification of in vivo tumor pH revealed that MCT4-expressing tumors maintain an alkaline pHi while the extracellular pH is very acidic, compared to tumors lacking MCT4 [29]. Pharmaceutical companies are actually working on the synthesis of specific inhibitors of MCT4. Inducible shRNA to knock down either the MCT1 and MCT4 (that leads to a decrease in CD147 expression) or the chaperone CD147 (leading to a decrease in MCT1 and MCT4 expression) significantly reduced glycolytic flux and tumor proliferation of human colon adenocarcinoma LS174 cell line. Finally, using Zinc finger nuclease technology we succeed in silencing both MCT1 and MCT4, keeping high levels of CD147, which demonstrate that the protumoral fonction of CD147 is to control the glycolytic rate of tumors *via* MCT1 and MCT4 activities [28]. Consequently CD147 appears as a good candidate to block lactic acid export.

In light of the prominent Warburg-effect, the inhibition of glycolytic enzymes is extensively studied to reduce ATP production in tumor cells. We focused our interest on the glyceraldehyde-3 phosphate dehydrogenase (GAPDH), a target of *Myc* (2) and HIF-1 [30–32], as increased mRNA expression of GAPDH was correlated with reduced overall survival and relapse-free survival for breast cancer patients [33]. However, the mechanism by which GAPDH expression is associated with tumor aggressivness remains unclear. In addition to its glycolytic function that is to catalyze a key step producing the essential energetic cofactor NADH, GAPDH is a multifunctional protein [34,35] overexpressed in the vast majority of human tumors [36]. In most human tumor cells lines as also in primary B lymphoma cells isolated from Eμ-*Myc* transgenic mice, we recently established that GAPDH overexpression activates the PI3 K/Akt signaling pathway, thereby increasing the expression of the anti-apoptotic Bcl-xL, a process prevented by specific inhibition of GAPDH

with koningic acid. As a consequence, *Myc*-driven B lymphoma expressing high levels of active GAPDH are display an aggressive phenotype in vivo. An extensive characterization of the koningic acid on *Myc*-driven B lymphoma growth from our laboratory will be presented in the near future.

The specificity of certain proteins to tumor growth and survival provide the means to develop pharmacological inhibitors and/or inhibitory antibodies to continue the pursuit of our goal in the selective killing of tumor cells. Our presentation at this symposia and this brief manuscript aim to highlight and synthesize recent advances that present some of the more promising anticancer strategies to focus on in the future.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgements

JC was supported by the "Association pour la Recherche sur le Cancer" (ARC).

References

[1] Warburg O. On respiratory impairment in cancer cells. Science 1956;124(3215):269–70.
[2] Osthus RC, Shim H, Kim S, et al. Deregulation of glucose transporter 1 and glycolytic gene expression by c-Myc. J Biol Chem 2000;275(29):21797–800.
[3] Shim H, Dolde C, Lewis BC, et al. c-Myc transactivation of LDH-A: implications for tumor metabolism and growth. Proc Natl Acad Sci U S A 1997;94(13):6658–63.
[4] Kroemer G, Pouyssegur J. Tumor cell metabolism: cancer's Achilles' heel. Cancer Cell 2008;13(6):472–82.
[5] Semenza GL. Targeting HIF-1 for cancer therapy. Nat Rev Cancer 2003;3(10):721–32.
[6] Dang CV, Kim JW, Gao P, Yustein J. The interplay between MYC and HIF in cancer. Nat Rev Cancer 2008;8(1):51–6.
[7] Faubert B, Boily G, Izreig S, et al. AMPK is a negative regulator of the warburg effect and suppresses tumor growth in vivo. Cell Metab 2013;17(1):113–24.
[8] Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci U S A 1995;92(12):5510–4.
[9] Berra E, Roux D, Richard DE, Pouyssegur J. Hypoxia-inducible factor-1 alpha (HIF-1 alpha) escapes O(2)-driven proteasomal degradation irrespective of its subcellular localization: nucleus or cytoplasm. EMBO Rep 2001;2(7):615–20.
[10] Epstein AC, Gleadle JM, McNeill LA, et al. C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. Cell 2001;107(1):43–54.
[11] Kaelin Jr WG, Ratcliffe PJ. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. Mol Cell 2008;30(4):393–402.
[12] Lando D, Peet DJ, Gorman JJ, Whelan DA, Whitelaw ML, Bruick RK. FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. Genes Dev 2002;16(12):1466–71.
[13] Semenza GL, Nejfelt MK, Chi SM, Antonarakis SE. Hypoxia-inducible nuclear factors bind to an enhancer element located 3' to the human erythropoietin gene. Proc Natl Acad Sci U S A 1991;88(13):5680–4.
[14] Chiche J, Brahimi-Horn MC, Pouyssegur J. Tumour hypoxia induces a metabolic shift causing acidosis: a common feature in cancer. J Cell Mol Med 2010;14(4):771–94.
[15] Trastour C, Benizri E, Ettore F, et al., HIF-1alpha CA IX staining in invasive breast carcinomas: prognosis and treatment outcome. Int J Cancer 2007;120(7):1451–8.
[16] Jacquemier J, Ginestier C, Bertucci F, Jauffret EC, Geneix J, Birnbaum D. Tissue microarrays a powerful tool in transfer and quality control in oncology. Bull Cancer 2003;90(1):31–8.
[17] van Uden P, Kenneth NS, Webster R, Muller HA, Mudie S, Rocha S. Evolutionary conserved regulation of HIF-1beta by NF-kappaB. PLoS Genet 2011;7(1):e1001285.
[18] Rius J, Guma M, Schachtrup C, et al. NF-kappaB links innate immunity to the hypoxic response through transcriptional regulation of HIF-1alpha. Nature 2008;453(7196):807–11.
[19] Mazure NM, Chen EY, Laderoute KR, Giaccia AJ. Induction of vascular endothelial growth factor by hypoxia is modulated by a phosphatidylinositol 3-kinase/Akt signaling pathway in Ha-ras-transformed cells through a hypoxia inducible factor-1 transcriptional element. Blood 1997;90(9):3322–31.
[20] Choo AY, Yoon SO, Kim SG, Roux PP, Blenis J. Rapamycin differentially inhibits S6Ks and 4E-BP1 to mediate cell-type-specific repression of mRNA translation. Proc Natl Acad Sci U S A 2008;105(45):17414–9.
[21] Parks SK, Chiche J, Pouyssegur J. pH control mechanisms of tumor survival and growth. J Cell Physiol 2011;226(2):299–308.
[22] Chiche J, Ilc K, Brahimi-Horn MC, Pouyssegur J. Membrane-bound carbonic anhydrases are key pH regulators controlling tumor growth and cell migration. Adv Enzyme Regul 2010;50(1):20–33.
[23] Chiche J, Ilc K, Laferriere J, et al. Hypoxia-inducible carbonic anhydrase IX and XII promote tumor cell growth by counteracting acidosis through the regulation of the intracellular pH. Cancer Res 2009;69(1):358–68.
[24] Pouyssegur J, Franchi A, Pages G. pHi, aerobic glycolysis and vascular endothelial growth factor in tumour growth. Novartis Found Symp 2001;240:186–96, discussion 96–8.
[25] Morris JC, Chiche J, Grellier C, et al. Targeting hypoxic tumor cell viability with carbohydrate-based carbonic anhydrase IX and XII inhibitors. J Med Chem 2011;54(19):6905–18.
[26] Doyen J, Parks SK, Marcie S, Pouyssegur J, Chiche J. Knock-down of hypoxia-induced carbonic anhydrases IX and XII radiosensitizes tumor cells by increasing intracellular acidosis. Front Oncol 2013;2:199.
[27] 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(14):9030–7.
[28] Le Floch R, Chiche J, Marchiq I, et al. CD147 subunit of lactate/H+ symporters MCT1 and hypoxia-inducible MCT4 is critical for energetics and growth of glycolytic tumors. Proc Natl Acad Sci U S A 2011;108(40):16663–8.
[29] Chiche J, Le Fur Y, Vilmen C, et al. In vivo pH in metabolic-defective Ras-transformed fibroblast tumors: key role of the monocarboxylate transporter, MCT4, for inducing an alkaline intracellular pH. Int J Cancer 2012;130(7):1511–20.
[30] Graven KK, Yu Q, Pan D, Roncarati JS, Farber HW. Identification of an oxygen responsive enhancer element in the glyceraldehyde-3-phosphate dehydrogenase gene. Biochim Biophys Acta 1999;1447(2–3):208–18.
[31] Lu S, Gu X, Hoestje S, Epner DE. Identification of an additional hypoxia responsive element in the glyceraldehyde-3-phosphate dehydrogenase gene promoter. Biochim Biophys Acta 2002;1574(2):152–6.
[32] Dayan F, Roux D, Brahimi-Horn MC, Pouyssegur J, Mazure NM. The oxygen sensor factor-inhibiting hypoxia-inducible factor-1 controls expression of distinct genes through the bifunctional transcriptional character of hypoxia-inducible factor-1alpha. Cancer Res 2006;66(7):3688–98.
[33] Revillion F, Pawlowski V, Hornez L, Peyrat JP. Glyceraldehyde-3-phosphate dehydrogenase gene expression in human breast cancer. Eur J Cancer 2000;36(8):1038–42.
[34] Colell A, Green DR, Ricci JE. Novel roles for GAPDH in cell death and carcinogenesis. Cell Death Differ 2009;16(12):1573–81.
[35] Sirover MA. New nuclear functions of the glycolytic protein, glyceraldehyde-3-phosphate dehydrogenase, in mammalian cells. J Cell Biochem 2005;95(1):45–52.
[36] Altenberg B, Greulich KO. Genes of glycolysis are ubiquitously overexpressed in 24 cancer classes. Genomics 2004;84(6):1014–20.