Histol Histopathol (2018) 33: 655-663

http://www.hh.um.es

Histology and Histopathology

From Cell Biology to Tissue Engineering

Review

Potential role of NDRG2 in reprogramming cancer metabolism and epithelial-to-mesenchymal transition

Xiang-Liu Chen, Lan Lei, Lian-Lian Hong and Zhi-Qiang Ling

Zhejiang Cancer Institute, Zhejiang Cancer Hospital, Zhejiang Cancer Center, Gongshu District, Hangzhou, P.R. China

Summary. Epithelial-to-mesenchymal transition (EMT) allows a cell with epithelial characteristics to transdifferentiate into a cell with mesenchymal characteristics, which is recognized as a key priming event for the initiation and evolvement of cancer metastasis. Accumulating data has shown that aberrant cancer metabolism contributes to the execution of EMT and cancer metastasis through multiple pathological pathways. Recently, the N-MYC downstream-regulated gene 2 (NDRG2), as a tumor suppressor and metabolism-related gene in various cancers, has been widely noted. NDGR2 is associated with energy metabolism, especially glycose metabolism. Hence, we propose a hypothesis that EMT is repressed by NDRG2 via cancer metabolic reprogramming, and summarize the pathological processes and molecular pathways related to the regulation of NDRG2.

Key words: NDRG2 (N-MYC downstream-regulated gene 2), Tumor suppressor gene, EMT (Epithelial-to-mesenchymal transition), Metastasis, Metabolic reprogramming

Offprint requests to: Zhi-Qiang Ling, MD.,PhD, Professor, Zhejiang Cancer Institute, Zhejiang Cancer Hospital, Zhejiang Cancer Center. No.1 Banshan East Road, Gongshu District, Hangzhou 310022, P.R. China. e-mail: lingzq@zjcc.org.cn or lingzq@hotmail.com

DOI: 10.14670/HH-11-957

Introduction

In the 1920s, Otto Warburg initially reported that the majority of cancer cells increase glycolysis even in the presence of adequate oxygen (Warburg, 1956). This process was known as the "Warburg effect" and described the way by which cancerous cells utilize aerobic glycolysis from mitochondrial respiration. During the past decades, the metabolic abnormalities that participate in tumorigenesis and tumor progression have been identified as hallmark characteristics of cancer (Cantor and Sabatini, 2012). However, the mechanisms of metabolic reprogramming have not been fully described. NDRG2 may be considered a promising target in order to explore the link between metabolism and tumor progression, since there are several reports suggesting that the expression of NDRG2 is downregulated and/or absent in a variety of cancers. In addition, NDRG2 may be considered a metabolism-related gene involved in cellular metabolic processes, including glycolysis, glutaminolysis and fatty acid oxidation metabolism (Xu et al., 2015; Pan et al., 2017). Currently, cancer metastasis is the leading cause of the majority of cancer-associated mortalities. EMT exerts an essential effect on the occurrence and progression of cancer metastasis. Intriguingly, there is an association between aberrant metabolism and EMT. Therefore, this review aims to investigate the recent reports regarding NDGR2, aberrant metabolism and EMT in cancer. The present study further discusses the molecular mechanism by which NDRG2 regulates EMT via reprogramming of the cancer cell metabolism. The understanding of the explicit carcinogenic pathways may provide new

therapeutic targets for cancer.

NDRG2 acts as a molecular rheostat of EMT

The NDRG family comprises 4 members, namely NDRG1-4, which are located on chromosomes 8q24.3, 14q11.2, 20q11.21-11.23 and 16q21-q22.1, respectively. The NDRG proteins are characterized by an esterase/lipase/thioesterase active site serine and a α/β hydrolase fold regime, sharing approximately 57-65% amino acid identity (Fig. 1) (Hu et al., 2016; Lee et al., 2016a,b). Phylogenetic analyses indicated that NDRG1 and NDRG3 could be classified into one subfamily, whereas NDRG2 and NDRG4 were included in a separate subfamily. Oncogenic and/or tumor suppressive effects have been documented with regard to the low/high expression of NDRG proteins that impact on key hallmark traits of carcinogenesis, such as proliferation, differentiation, apoptosis, migration, invasion and stress responses (Melotte et al., 2010).

NDRG2 was initially identified and cloned by subtractive hybridization (Deng et al., 2003). NDRG2 is expressed in a wide range of tissues and organs, including heart, brain, liver, kidney, cartilage, gut, skeletal muscle and chorion. The expression of NDRG2 correlated with several human primary tumors, and was down-regulated in breast cancer (Ma et al., 2012; Kim et al., 2014a-c), hepatocellular carcinoma (Guo et al., 2013; Cao et al., 2014; Wang et al., 2014), glioblastoma (Deng et al., 2003), lung cancer (Wang et al., 2012), colorectal cancer (Shen et al., 2014), oral squamous cell carcinoma (OSCC) (Tamura et al., 2017), prostate cancer (Gao et al., 2011; Yu et al., 2015) , gallbladder carcinoma (Lee et al., 2015) and gastric cancer (Tao et al., 2013) (Table 1). Thus, accumulating evidence indicates that NDRG2 may be a tumor suppressor gene. Furthermore, the down-regulation of NDRG2 is associated with malignant clinical features and increased pathological grade. NDRG2 can act as a biomarker for the prediction of an aggressive phenotype, tumor recurrence, and patient prognosis. The NDRG2 promoter undergoes epigenetic silencing by methylation in the majority of primary tumors, which can give rise to resistance against chemotherapy. Although the biological functions of NDRG2 in tumors have been thoroughly studied, limited information is available with regard to the contribution of NDRG2 in reprogramming metabolism and regulating the EMT.

The novel function of NDRG2: modulators of metabolic reprogramming

Abnormal metabolism is currently regarded as the new malignant phenotype of cancerous cells. The regulatory roles of NDRG2 in tumor inhibition, particularly in metabolic rewiring, remain largely unclear. Thus, the present study notably focused on previous reports regarding the regulation of NDRG2 in the process of energy metabolism in cancer cells. A well-known metabolic abnormality in tumor cells is the Warburg effect that is characterized by increased glycolytic flux from pyruvate to lactate. In addition, aberrant glutamine and lipid metabolism are further involved in the occurrence and development of tumor cells via ATP production (Currie et al., 2013).

The transportation of glucose across the plasma

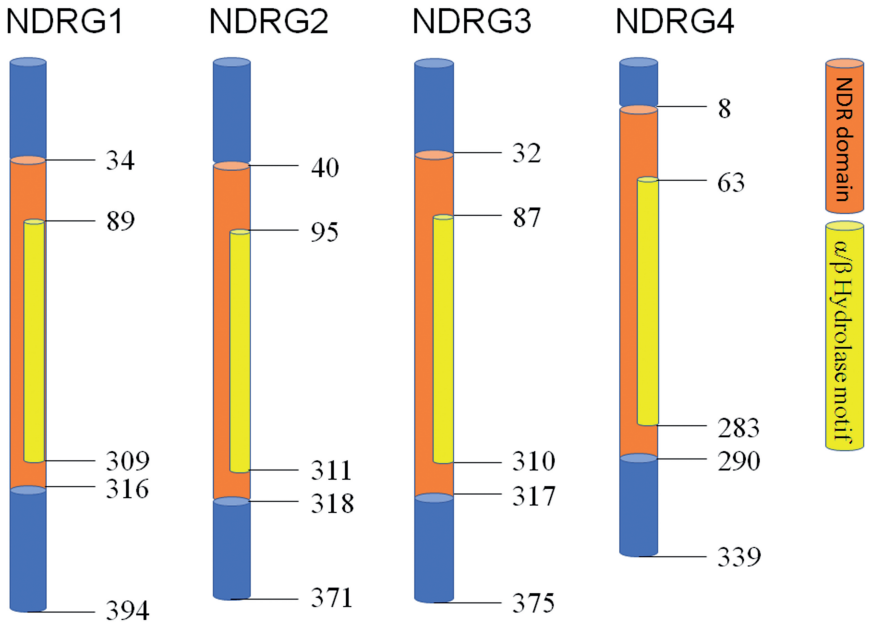

Fig. 1. Stucture of the human NDRG protein sequences. Illustrated are the different domains present in the NDRG family members. Depicted are the NDR domain (as defined by the Pfam database), and regions altered in different isoforms (as defined by UniProt). The NDRG family members are 53-65% homologous to each other and share the conserved NDR domain, which includes the alpha/beta hydrolase motif (Lee et al., 2015; Melotte et al., 2010).

membrane is the first rate-limiting step for glucose metabolism and is executed by glucose transporter proteins (GLUTs). Among the four NDRG members, NDRG2 was the first to be regarded as a metabolic programming protein. NDRG2 inhibits the transportation of glucose by promoting GLUT1 protein degradation without regulating GLUT1 mRNA level. Furthermore, the high expression of NDRG2 is associated with a better prognosis in breast carcinoma (Ma et al., 2014). Secondly, Xu et al. reported that NDRG2 inhibited the expression of c-MYC by suppressing β-catenin levels, which could activate the transcription of the c-MYC gene in the nucleus. Subsequently, the transcription factor c-MYC directly regulates various glucose and glutamine metabolism genes, namely GLUT1, hexokinase 2 (HK2), pyruvate kinase M2(PKM2), lactate dehydrogenase A (LDHA), glutaminase 1 (GLS1) and glutamine transporter ASC amino-acid transporter 2(ASCT2). Consequently, NDRG2 can markedly suppress these glycolytic and glutaminolytic targets by the MYC-mediated metabolic reprogramming in colorectal cancer (Xu et al., 2015).

The Wnt/β-catenin and the PI3K-Akt-mTOR pathways are both significant regulatory pathways that can increase the Warburg effect and the anabolic synthesis (Chafey et al., 2009; Ward and Thompson, 2012) in cancer cells. NDRG2 has been shown to participate in the two processes. The reduced expression of NDRG2 was to activate the Wnt signaling pathway, which is a main contributor in the tumorigenesis of hepatoblastoma (Gödeke et al., 2016). Moreover, the Wnt/β-catenin pathway directly targets pyruvate dehydrogenase kinase 1 (PDK1) in order to direct the cancer phenotype of the Warburg effect (Pate et al., 2014). Indeed, c-MYC was identified as a downstream target gene of the Wnt/β-catenin pathway, and the expression of this gene was downregulated by inhibition of the Wnt/β-catenin pathway (Sansom et al., 2007). Thus, NDRG2 may attenuate Wnt-driven Warburg metabolism. In contrast to these observations, NDRG2 is capable of recruiting PP2A in order to induce PTEN-Ser380/Thr382/Thr383 dephosphorylation, and subsequently suppresses PI3K-AKT activation in the adult T-cell leukemia-lymphoma and OSCC (Nakahata et al., 2014; Tamura et al., 2017). Taken collectively, the studies suggest that NDRG2 functions as a metabolic gene via repression of the PI3K-AKT pathway that in turn inhibits the progression of cancer.

With regard to lipid metabolism, NDRG2 impairs the activation of fatty acid oxidation (FAO) in hepatoma cells. This supports ATP and NADPH purveyance via suppression of the AMPK/ACC pathway in the absence of glucose (Zhang et al., 2017). NDRG2 overexpression reduces the expression level of peroxisome proliferator-activated receptors alpha (PPARα) - a main transcription factor that synergistically controls the FAO genes, carnitine palmitoyltransferase 1A (CPT1A) - the rate-limiting enzyme translocating acyl-coenzyme A into mitochondria to complete FAO, as well as acyl-CoA dehydrogenase, C-4 to C-12 straight chain (ACADM) - an important enzyme that regulates the initial step of FAO (Pan et al., 2017). However, the molecular mechanisms regarding these processes are not fully explored.

Metabolic rewiring induces EMT activation

EMT acts as the key driver of cancer cell metastasis. This process is orchestrated by a network of transcription factors (EMT-TFs), including Snail, Twist, Zeb, and the Fox family of proteins (Ye and Weinberg, 2015). The mechanisms by which EMT-TFs directly or indirectly inhibit the key epithelial marker E-cadherin, have been described in detail in a previous study

Table 1. The role of NDGR2 as tumor suppressor gene in cancer.

Tumor type	Expression	Pathway	Function	Reference
Colorectal cancer	Downregulated; Its expression in cancer tissues correlates with good prognosis	TGF-β	Invasion and metastasis	Shen et al., 2014
Hepatocellular carcinoma		p53	Apoptosis	Guo et al., 2013; Wang et al., 2014; Cao et al., 2014
		ERK1/2, gp130/STAT3	Invasion and metastasis	
Glioblastoma		--	Proliferation	Deng et al., 2003
Breast cancer		NF-κB/COX-2, STAT3/Snail	Migration and invasion	Kim et al., 2014a-c; Ma et al., 2012
		LKB1-AMPK	Apoptosis	
		p53 , VRGF, HIF-α	Proliferation, angiogenesis	
Lung cancer		--	Metastasis	Wang et al., 2012
Colorectal cancer		TGF-β	EMT, metastasis	Shen et al., 2014
OSCC		PI3K/AKT, NF-κB	Metastasis and invasion	Tamura et al., 2017
Prostate cancer		IL-8, MMP2, MMP9	Metastasis and invasion	Gao et al., 2011; Yu et al., 2015
		AR signaling pathway	Growth	
Gallbladder carcinoma		MMP19-Slug	EMT	Lee et al., 2015
Gastric cancer		--	Apoptosis and invasion	Tao et al., 2013

(Lamouille et al., 2014). Conversely, the metabolic programming that is responsible for the EMT phenotype of an epithelial cell has been poorly understood. Recent evidence indicates that the association between metabolism and EMT is mutual, whereas alterations in the metabolic pathways can further promote the progression of EMT under certain conditions (Sciacovelli and Frezza, 2017). This part of the review mainly describes how the abnormal levels of glucose, and the deregulated pathways of glutaminolysis and fatty acid metabolism are linked with the induction of EMT.

Glycolysis

Aerobic glycolysis is the main characteristic metabolic reprogramming of cancer cells (Pavlova et al., 2016). HK2 catalyzes the conversion of glucose to glucose-6-phosphate (G-6-P) and is the first rate-limiting enzyme of glycolysis. This enzyme is usually up-regulated in cancer (Palmieri et al., 2009). It has been recently reported that 2-deoxyglucose (2DG), a competitive inhibitor of HK2, can attenuate the pro-metastatic phenotype, reversing angiogenesis and EMT, in a TGFβ-dependent manner (Bacci et al., 2016; Penny et al., 2016). This process has been reported in pancreatic ductal adenocarcinoma and ER+ breast cancer.

PKM2 controls the last rate-limiting step of glycolysis and catalyzes the final irreversible reaction of the conversion of phosphoenolpyruvate (PEP) to pyruvate that produces ATP. PKM2 has been shown to act as a potent EMT inducer. The overexpression of PKM2 is coupled with malignant clinicopathological features and unsatisfactory patient prognosis in various types of cancer, such as prostate cancer (Giannoni et al., 2015), hepatocellular carcinoma (Fan et al., 2014), colon cancer (Yang et al., 2014) and cervical carcinoma (Cheng and Hao, 2016, 2017). Although previous reports suggested that the metabolic function of PKM2 is required for aerobic glycolysis, this enzyme can translocate into the nucleus, playing a role in nonmetabolic transcriptional regulation. Fan et al. demonstrated that nuclear translocation of PKM2 is essential for the EGF (epithelial growth factor)-induced EMT activation, which occurs via the transcription of β-catenin that promotes expression of Snail and Vimentin and reduces expression of E-cadherin (Fan et al., 2014). In addition, PKM2 can bind to TGF-β-induced factor homeobox 2(TGIF2), which recruits histone deacetylase 3 to the promoter of E-cadherin and subsequently decreases its expression (Hamabe et al., 2014). Moreover, overexpression of PKM2 increases the expression of N-cadherin, Snail2, MMP-2 and MMP-9 and conversely suppresses the expression of E-cadherin by regulating the STAT3-related signaling pathway to facilitate migration (Yang et al., 2014). The inhibition of mTOR/p70s6k signaling downregulates PKM2 expression and abolishes TGF-β1-induced EMT (Cheng and Hao, 2016, 2017).

LDHA is the main regulator of aerobic glycolysis and catalyzes the irreversible convertion of pyruvate to lactate. A multitude of studies indicated that the attenuation of LDHA inhibits the invasive and metastatic properties of cancer cells (Arseneault et al., 2013; Jin et al., 2017). Initially, Sheng et al. demonstrated that knockdown of LDHA leads to an increase in E-cadherin and a decrease in focal adhesion kinase (FAK), MMP2 and VEGF levels, all of which are involved in EMT (Sheng et al., 2012). In addition, a recent study reported that LDHA silencing in bladder cancer cells can significantly inhibit EMT as demonstrated by the upregulation of the epithelial marker E-cadherin and the downregulation of the mesenchymal markers Snail, N-cadherin, Vimentin and fibronectin at the mRNA and protein levels (Jiang et al., 2016). Despite several studies demonstrating that LDHA can directly regulate the process of EMT, its product lactate and the formation of reactive oxygen species (ROS) further participate in this phenotype. In contrast to these observations, lactate has been shown to upregulate TGF-β2 expression (Seliger et al., 2013), which subsequently promotes a mesenchymal pro-migration phenotype and elicits MMP-2 expression, ECM remodeling and metastasis in glioma cells (Wick et al., 2006). In addition, LDHA attenuation leads to a redirection of glucose flux to the TCA cycle for OXPHOS subsequent induction of ROS. Enhanced ROS plays an inhibitory role in tropomyosin-mediated cell migration and cytoskeletal remodeling, which suppresses the EMT phenotype and metastasis (Arseneault et al., 2013).

Glutaminolysis

Elevated glycolysis is the most well-known feature associated with malignancies. However, glutaminolysis is another indispensable characteristic for cancer cells, and has been frequently correlated with EMT progression. GLS is the pivotal enzyme for the catalysis of glutamine to glutamate in glutaminolysis. This enzyme is encoded by two types of genes in mammalian cells, namely GLS1 and GLS2. GLS1 is up-regulated in cancer development and progression and acts as an oncogene in diverse tumors. Inhibition of glutaminolysis by GLS1 shRNA damages Snail-dependent EMT and metastasis via the inhibition of TGF-β and Wnt3a expression (Lee et al., 2016a,b). In contrast to its oncogenic function, GLS2 further exhibits tumor suppressive activity and is inversely associated with stage, tumor size and prognosis in hepatocellular carcinoma (Liu et al., 2014). A study has highlighted that GLS2 can exert non-glutaminolysis-associated function in order to repress EMT and malignant phenotype through the Dicer-miR-34a-Snail axis (Kuo et al., 2016). Thus, it is speculated that the roles of GLS1 and GLS2 on the process of EMT are context-dependent, and additional work is required to elucidate the specific differences between them. The overexpression of GLS1 decreases the levels of its substrate glutamine and plays a part in the suppression of TGF-β-induced EMT in liver tissues (Shrestha et al., 2016).

Fatty acid metabolism

Although, glucose and glutamine alterations have been extensively studied in the context of cancer metabolism, several reports have recently demonstrated the connection between lipid metabolism and EMT.

For example, the inhibition of the lipogenic enzyme fatty acid synthase (FASN) results in the reversal of the EMT phenotype in breast cancer via the suppression of vimentin, N-cadherin and fibronectin (Gonzalez-Guerrico et al., 2016). In addition, the elevated free fatty acid uptake exacerbates the EMT phenotype via the activation of the Wnt and TGF-β signaling pathways (Nath et al., 2015). It is important to note that the upregulation of acetyl-CoA synthetase (ACSL) and stearoyl-CoA desaturase (SCD) activates AMPK signaling in order to trigger an EMT program which is responsible for cancer invasion and migration (Sanchez-Martinez et al., 2015). Additional enzymes that are involved in lipid metabolism have been identified as EMT regulators. The silencing of acetyl-CoA carboxylase 2 (ACC2) reverses the EMT transition caused by high glucose, triglyceride deposit and accumulation of malonyl CoA content in the kidney (Xu et al., 2014). In addition, ATP citratelyase (ACL) knockdown increases expression of the epithelial markers (E-cadherin, ZO-1) and decreases expression of the mesenchymal marker vimentin in lung cancer cells undergoing a reversal of EMT (Hanai et al., 2012). Furthermore, both arachidonic acid and linoleic acid promote the process of EMT via the activation of FAK, SRC and NF-κB in human mammary non-tumorigenic epithelial cells (Martinez-Orozco et al., 2010; Espinosa-Neira et al., 2011).

CLUT expression and acidic environment

In addition to the aforementioned enzymes, GLUTs contribute a significant role in cancer cell metabolic reprogramming and the development of EMT. GLUTs transport glucose across the plasma membrane and are considered the enzymes responsible for the first rate-limiting step of glucose metabolism. A substantial connection between GLUT1 expression and metastasis has been supported by previous studies. For example, Zuo et al. demonstrated correlations of GLUT1 expression and vimentin, N-cadherin and E-cadherin (Zuo et al., 2016) that are consistent with the study that demonstrated the down-regulation of E-cadherin by GLUT1 (Mayer et al., 2013). The up-regulated GLUT1 can further increase the expression and activity of MMP-2 to promote invasiveness of cancer (Ito et al., 2004). Interestingly, an additional isoform of glucose transporter family members, GLUT3 augmented glucose consumption and correlated with EMT signatures in the process of lung tumor progression (Masin et al., 2014). Moreover, both GLUT1 and GLUT3 promoted glucose uptake that was shown to induce EMT by stabilization of Snail (Park et al., 2010).

Nevertheless, EMT formation is not only affected by aerobic glycolysis, but as well the low pHe environment, which results from the final glycolytic metabolite lactate and a variety of proton exchangers. Mounting evidence has revealed that the presence of an acidic environment confers a metastatic advantage to cancer cells (Peppicelli et al., 2014a,b; Suzuki et al., 2014). Silvia et al. highlighted that an acidic pHe medium in melanoma cells up-regulates EMT markers, including N-cadherin, vimentin and Twist transcription. This phenomenon was proven to be NF-κB-dependent (Peppicelli et al., 2014a,b). The effect of low pHe in lung carcinoma induced the expression of Zeb2, Twist1 and Twist2 at the mRNA level and promoted the invasive and metastatic activities of the cells (Suzuki et al., 2014). Similar findings were reported for human melanoma cells (Suzuki et al., 2014). Accordingly, the studies illustrate that low pHe generates an acidic microenvironment and elicits EMT in cancer metastasis. Acidosis is also associated with apoptosis, angiogenesis and extracellular matrix (ECM) formation in cancer cells (Huang et al., 2017).

NDRG2 inhibits EMT via the regulation of cancer metabolism

With regard to cancer metastasis, a limited number of studies have reported that NDRG2 regulated the process of EMT via GSK-3β-Snail, TGF-β, MMP-19-Slug and STAT3-Snail signaling in colon, colorectal, gallbladder and breast cancer, respectively (Kim et al., 2013; Kim et al., 2014a-c; Shen et al., 2014; Lee et al., 2015; Tamura et al., 2017). Tamura et al. recently highlighted that high expression of NDRG2 induced inactivation of NF-κB and PI3K/AKT signaling pathways via the dephosphorylation of the C-terminal domain of PTEN, and the inhibition of the process of EMT in OSCC (Tamura et al., 2017). Nonetheless, the direct molecular mechanisms by which NDRG2 inhibits EMT have not yet been fully clarified.

The present study clearly indicates that loss of NDRG2 contributes to induction of metabolic reprogramming, notably the Warburg effect, via a series of signaling pathways (Xu et al., 2015; Lee et al., 2016a,b). Concomitanly, the alteration of the cellular metabolism can intervene with the process of EMT that endows cancer cells with invasive and migratory capacities, and facilitates the tumor dissemination and the metastatic process (Huang et al., 2017; Sciacovelli and Frezza, 2017). Taken collectively, the study demonstrates that NDRG2 is responsible for the inhibition of EMT via the regulation of cancer cell metabolism. Previous studies have concluded that the deficiency of NDRG2 induces the activation of Wnt/β-catenin, PI3K-Akt-mTOR and c-MYC pathways, which in turn stimulate the Warburg metabolism of cancer cells. However, MYC and HIF-1 cooperatively counterbalance the reprogramming of cancer metabolism. MYC activates the Warburg effect by

inducing HK2, phosphofructokinase (PFKM), PKM2, enolase 1 (ENO1), as well as mitochondrial biogenesis (Dang et al., 2009). Like MYC, NDRG2 also could attenuate human renal cancer cell invasion and proliferation by inhibiting HIF-1α mediated processes, which are responsible for upregulating glucose transporters and various glycolytic enzymes to shift oxidative phosphorylation to the glycolytic metabolism (Gao et al., 2013; Semenza, 2013). Similarly, NDRG2 restrains glycolytic metabolic program, during which glycolytic enzymes throught their metabolic or nonenzymatic activity, induce EMT and drive tumor metastasis in various cancers (Sajnani et al., 2017; Xu et al., 2017). With regard to glutaminolysis and lipid metabolism, NDRG2 can further exert a negative impact on the regulation of GLS1, ASCT2 and fatty acid oxidation, which can trigger the activation of EMT-TFs and promote the EMT process (Xu et al., 2015; Morandi et al., 2017; Pan et al., 2017). Therefore, NDRG2 can play a key role in the occurrence and progression of EMT by targeting the cellular metabolism that determines the energy production of the cell.

Cancer cells adapt to the adverse microenvironment with elevated glucose uptake and glycolysis, thus resulting in the production of an acidic environment. In the present study, loss of NDRG2 was further demonstrated to increase the expression of correlative glycolytic enzymes and GLUTs, which indicates that excessive lactate is produced by immoderate glycolysis. Finally, the acidic microenvironment represents a direct contributor to the process of EMT. In conclusion, a certain number of studies have suggested that NDRG2 regulates metabolic rewiring and subsequently induces EMT (Fig. 2).

Conclusion

A link between NDRG2 and epithelial-mesenchymal transition has not only given us a better comprehension of the role of metabolic reprogramming in cancer metastasis, but also has provided further insight into the tumor-suppressive function of NDRG2 through a novel regulatory mechanism. In this paper, we firstly propose that rewiring energy metabolism connects NDRG2 to the occurrence and development of EMT, which drives the invasive ability of malignant tumors. Deciphering the connection between NDGR2 and EMT may serve as the crucial factor in determining the therapeutic point that exerts maximum drug efficacy. At the same time, metabolic reprogramming has been considered as an emerging target for cancer therapy. Herein, applying NDRG2 alone or in combination with anti-glycolytic

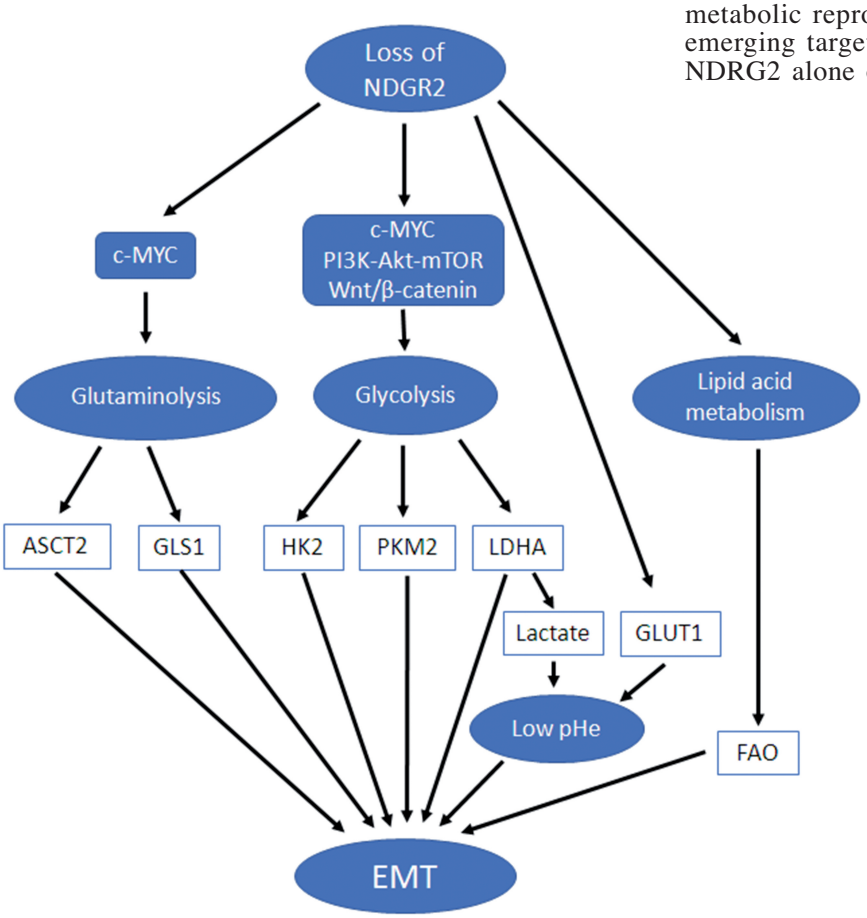

Fig. 2. The molecular mechanism of NDRG2 regulating the process of EMT though rewiring metabolism. The energy metabolism acts as a crosstalk between NDRG2 and EMT.

agent may validly and synergistically treat metastasis of cancer cells and ameliorate prognosis of patients.

Acknowledgements. This study was supported by National Natural Science Foundation of China (81372332 and 81572822), Key Projects from the Natural Science Foundation of Zhejiang Province of China (LZ13H160002 and LZ18H160002), Zhejiang Provincial Program for the Cultivation of High-Level Innovative Health Talents (Zjwjw2014-108), the Major Training Personnel from Zhejiang Provincial Program for the Training and Development Project for 151 Talents (Zjhrss2014-150), the Analysis Project from Zhejiang Provincial Public Scientific and Technological Innovation Platform (2015C37043), Science Foundation of National Health and National Family Planning Commission-Major Scientific and Technological Project for Medicine of Zhejiang Provincial Medicine and Hygiene (WKJ-ZJ-1505).

Ethics approval and consent to participate. This study was approved by the Institutional Review Board of Zhejiang Cancer Hospital (2013-03-79 and 2015-02-125).

Conflict of interest. No potential conflicts of interest were disclosed.

References

Arseneault R., Chien A., Newington J.T., Rappon T., Harris R. and Cumming R.C. (2013). Attenuation of LDHA expression in cancer cells leads to redox-dependent alterations in cytoskeletal structure and cell migration. Cancer Lett. 338, 255-266.

Bacci M., Giannoni E., Fearns A., Ribas R., Gao Q., Taddei M.L., Pintus G., Dowsett M., Isacke C.M., Martin L.A., Chiarugi P. and Morandi A. (2016). miR-155 drives metabolic reprogramming of ER+ breast cancer cells following long-term estrogen deprivation and predicts clinical response to aromatase inhibitors. Cancer Res. 76, 1615-1626.

Cantor J.R. and Sabatini D.M. (2012). Cancer cell metabolism: one hallmark, many faces. Cancer Discov. 2, 881-898.

Cao W, Zhang J.L., Feng D.Y., Liu X.W., Li Y., Wang L.F., Yao L.B., Zhang H. and Zhang J. (2014). The effect of adenovirus-conjugated NDRG2 on p53-mediated apoptosis of hepatocarcinoma cells through attenuation of nucleotide excision repair capacity. Biomaterials 35, 993-1003.

Chafey P., Finzi L., Boisgard R., Caüzac M., Clary G., Broussard C., Pégorier J.P., Guillonneau F., Mayeux P., Camoin L., Tavitian B., Colnot S. and Perret C. (2009). Proteomic analysis of beta-catenin activation in mouse liver by DIGE analysis identifies glucose metabolism as a new target of the Wnt pathway. Proteomics 9, 3889-3900.

Cheng K. and Hao M. (2016). Metformin Inhibits TGF-β1-induced epithelial-to-mesenchymal transition via PKM2 relative-mTOR/p70s6k signaling pathway in cervical carcinoma cells. Int. J. Mol. Sci. 17, 2000.

Cheng K.Y. and Hao M. (2017). Mammalian target of rapamycin (mTOR) regulates transforming growth factor-β1 (TGF-β1)-induced epithelial-mesenchymal transition via decreased pyruvate kinase M2 (PKM2) expression in cervical cancer cells. Med. Sci. Monit. 23, 2017-2028.

Currie E., Schulze A., Zechner R., Walther T.C. and Farese R.V. Jr (2013). Cellular fatty acid metabolism and cancer. Cell. Metab. 18, 153-161.

Deng Y., Yao L., Chau L., Ng S.S., Peng Y., Liu X., Au W.S., Wang J., Li F., Ji S., Han H., Nie X., Li Q., Kung H.F., Leung S.Y. and Lin M.C. (2003). N-Myc downstream-regulated gene 2 (NDRG2) inhibits glioblastoma cell proliferation. Int. J. Cancer 106, 342-347.

Dang C., Le A. and Gao P. (2009). MYC-induced cancer cell energy metabolism and therapeutic opportunities. Clin. Cancer Res. 1, 6479-6483.

Espinosa-Neira R., Mejia-Rangel J., Cortes-Reynosa P. and Salazar E.P. (2011). Linoleic acid induces an EMT-like process in mammary epithelial cells MCF10A. Int. J. Biochem. Cell. Biol. 43, 1782-1791.

Fan F.T., Shen C.S., Tao L., Tian C., Liu Z.G., Zhu Z.J., Liu Y.P., Pei C.S., Wu H.Y., Zhang L., Wang A.Y., Zheng S.Z., Huang S.L. and Lu Y. (2014). PKM2 regulates hepatocellular carcinoma cell epithelial-mesenchymal transition and migration upon EGFR activation. Asian Pac. J. Cancer Prev. 15, 1961-1970.

Gao L., Wu G.J., Liu X.W., Zhang R., Yu L., Zhang G., Liu F., Yu C.G., Yuan J.L., Wang H. and Yao L.B. (2011). Suppression of invasion and metastasis of prostate cancer cells by overexpression of NDRG2 gene. Cancer Lett. 310, 94-100.

Gao L., Wu G., Liu B., Shen M., Pan T., Yu C., Wang Q., Ru Y., Liu X,.Niu T.,Wang G., Wei M., Li R., Yao L., Wang H. and Li X. (2013). Up-regulation of pVHL along with down-regulation of HIF-1α by NDRG2 expression attenuates proliferation and invasion in renal cancer cells. PLoS One 8, e84127.

Giannoni E., Taddei M.L., Morandi A., Comito G., Calvani M., Bianchini F., Richichi B., Raugei G., Wong N., Tang D. and Chiarugi P. (2015). Targeting stromal-induced pyruvate kinase M2 nuclear translocation impairs oxphos and prostate cancer metastatic spread. Oncotarget 6, 24061-24074.

Gödeke J., Luxenburger E., Trippel F., Becker K., Häberle B., Müller-Höcker J., von Schweinitz D. and Kappler R. (2016). Low expression of N-myc downstream-regulated gene 2 (NDRG2) correlates with poor prognosis in hepatoblastoma. Hepatol. Int. 10, 370-376.

Gonzalez-Guerrico A.M., Espinoza I., Schroeder B., Park C.H., Kvp C.M., Khurana A., Corominas-Faja B., Cuyàs E., Alarcón T., Kleer C., Menendez J.A. and Lupu R. (2016). Suppression of endogenous lipogenesis induces reversion of the malignant phenotype and normalized differentiation in breast cancer. Oncotarget 7, 71151-71168.

Guo Y., Ma J., Wu L., Wang Q., Li X., Li X., Zhang Y., Zhang J., Yao L., Zhang J. and Liu W. (2013). Hyperthermia-induced NDRG2 upregulation inhibits the invasion of human hepatocellular carcinoma via suppressing ERK1/2 signaling pathway. PLoS One 8, e61079.

Hamabe A., Konno M., Tanuma N., Shima H., Tsunekuni K., Kawamoto K., Nishida N., Koseki J., Mimori K., Gotoh N., Yamamoto H., Doki Y., Mori M. and Ishii H. (2014). Role of pyruvate kinase M2 in transcriptional regulation leading to epithelial-mesenchymal transition. Proc. Natl. Acad. Sci. USA 111, 15526-15531.

Hanai J., Doro N., Sasaki A.T., Kobayashi S., Cantley L.C., Seth P. and Sukhatme V.P. (2012). Inhibition of lung cancer growth: ATP citrate lyase knockdown and statin treatment leads to dual blockade of mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase (PI3K)/AKT pathways. J. Cell. Physiol. 227, 1709-1720.

Hu W., Fan C., Jiang P., Ma Z., Yan X., Di S., Jiang S., Li T., Cheng Y. and Yang Y. (2016). Emerging role of N-myc downstream-regulated gene 2 (NDRG2) in cancer. Oncotarget 7, 209-223.

Huang R. and Zong X. (2017). Aberrant cancer metabolism in epithelial-mesenchymal transition and cancer metastasis: Mechanisms in cancer progression. Crit. Rev. Oncol. Hematol. 115, 13-22.

Ito H., Duxbury M., Benoit E., Farivar R.S., Gardner-Thorpe J., Zinner

M.J., Ashley S.W. and Whang E.E. (2004). Fibronectin-induced COX-2 mediates MMP-2 expression and invasiveness of rhabdomyosarcoma. Biochem. Biophys. Res. Commun. 318, 594-600.

Jiang F., Ma S., Xue Y., Hou J. and Zhang Y. (2016). LDH-A promotes malignant progression via activation of epithelial-to-mesenchymal transition and conferring stemness in muscle-invasive bladder cancer. Biochem. Biophys. Res. Commun. 469, 985-992.

Jin L., Chun J., Pan C., Alesi G.N., Li D., Magliocca K.R., Kang Y., Chen Z.G., Shin D.M., Khuri F.R., Fan J. and Kang S. (2017). Phosphorylation-mediated activation of LDHA promotes cancer cell invasion and tumour metastasis. Oncogene 36, 3797-3806.

Kim Y.J., Kang H.B., Yim H.S., Kim J.H. and Kim J.W. (2013). NDRG2 positively regulates E-cadherin expression and prolongs overall survival in colon cancer patients. Oncol. Rep. 30, 1890-1898.

Kim H.S., Kim M.J., Lim J., Yang Y., Lee M.S. and Lim J.S. (2014a). NDRG2 overexpression enhances glucose deprivation-mediated apoptosis in breast cancer cells via inhibition of the LKB1-AMPK pathway. Genes Cancer 5, 175-185.

Kim M.J., Kim H.S., Lee S.H., Yang Y., Lee M.S. and Lim J.S. (2014b). NDRG2 controls COX-2/PGE_2-mediated breast cancer cell migration and invasion. Mol. Cells 37, 759-765.

Kim M.J., Lim J., Yang Y., Lee M.S. and Lim J.S. (2014c). N-myc downstream-regulated gene 2 (NDRG2) suppresses the epithelial-mesenchymal transition (EMT) in breast cancer cells via STAT3/Snail signaling. Cancer Lett. 354, 33-342.

Kuo T.C., Chen C.K., Hua K.T., Yu P., Lee W.J., Chen M.W., Jeng Y.M., Chien M.H., Kuo K.T., Hsiao M. and Kuo M.L. (2016). Glutaminase 2 stabilizes Dicer to repress Snail and metastasis in hepatocellular carcinoma cells. Cancer Lett. 383, 282-294.

Lamouille S., Xu J. and Derynck R. (2014). Molecular mechanisms of epithelial-mesenchymal transition. Nat. Rev. Mol. Cell. Biol. 15, 178-196.

Lee D.G., Lee S.H., Kim J.S., Park J., Cho Y.L., Kim K.S., Jo D.Y., Song I.C., Kim N., Yun H.J., Park Y.J., Lee S.J., Lee H.G., Bae K.H., Lee S.C., Shim S., Kim Y.M., Kwon Y.G., Kim J.M., Lee H.J. and Min J.K. (2015). Loss of NDRG2 promotes epithelial-mesenchymal transition of gallbladder carcinoma cells through MMP-19-mediated Slug expression. J. Hepatol. 63, 1429-1439.

Lee G.Y., Chun Y.S., Shin H.W. and Park J.W. (2016a). Potential role of the N-MYC downstream-regulated gene family in reprogramming cancer metabolism under hypoxia. Oncotarget 7, 57442-57451.

Lee S.Y., Jeon H.M., Ju M.K., Jeong E.K., Kim C.H., Park H.G., Han S.I. and Kang H.S. (2016b). Dlx-2 and glutaminase upregulate epithelial-mesenchymal transition and glycolytic switch. Oncotarget 7, 7925-7939.

Liu J., Zhang C., Lin M., Zhu W., Liang Y., Hong X., Zhao Y., Young K.H., Hu W. and Feng Z. (2014). Glutaminase 2 negatively regulates the PI3K/AKT signaling and shows tumor suppression activity in human hepatocellular carcinoma. Oncotarget 5, 2635-2647.

Ma J., Liu W., Yan X., Wang Q., Zhao Q., Xue Y., Ren H., Wu L., Cheng Y., Li S., Miao L., Yao L. and Zhang J. (2012). Inhibition of endothelial cell proliferation and tumor angiogenesis by up-regulating NDRG2 expression in breast cancer cells. PLoS One 7, e32368.

Ma J., Liu W., Guo H., Li S., Cao W., Du X., Lei S., Hou W., Xiong L., Yao L., Li N. and Li Y. (2014). N-myc downstream-regulated gene 2 expression is associated with glucose transport and correlated with prognosis in breast carcinoma. Breast Cancer Res. 16, R27.

Martinez-Orozco R., Navarro-Tito N., Soto-Guzman A., Castro-Sanchez L. and Perez Salazar E. (2010). Arachidonic acid promotes epithelial-to-mesenchymal-like transition in mammary epithelial cells MCF10A. Eur. J. Cell Biol. 89, 476-488.

Masin M., Vazquez J., Rossi S., Groeneveld S., Samson N., Schwalie P.C., Deplancke B., Frawley L.E., Gouttenoire J., Moradpour D., Oliver T.G. and Meylan E. (2014). GLUT3 is induced during epithelial-mesenchymal transition and promotes tumor cell proliferation in non-small cell lung cancer. Cancer Metab. 2, 11.

Mayer A., Höckel M., Schlischewsky N., Schmidberger H., Horn L.C. and Vaupel P. (2013). Lacking hypoxia-mediated downregulation of E-cadherin in cancers of the uterine cervix. Br. J. Cancer 8, 402-408.

Melotte V., Qu X., Ongenaert M., van Criekinge W., de Bruïne A.P., Baldwin H.S. and van Engeland M. (2010). The N-myc downstream regulated gene (NDRG) family: diverse functions, multiple applications. FASEB J. 24, 4153-4166.

Morandi A., Taddei M., Chiarugi P. and Giannoni E. (2017). Targeting the metabolic reprogramming that controls epithelial-to-mesenchymal transition in aggressive tumors. Front. Oncol. 7, 40.

Nakahata S., Ichikawa T., Maneesaay P., Saito Y., Nagai K., Tamura T., Manachai N., Yamakawa N., Hamasaki M., Kitabayashi I., Arai Y., Kanai Y., Taki T., Abe T., Kiyonari H., Shimoda K., Ohshima K., Horii A., Shima H., Taniwaki M., Yamaguchi R. and Morishita K. (2014). Loss of NDRG2 expression activates PI3K-AKT signalling via PTEN phosphorylation in ATLL and other cancers. Nat. Commun. 5, 3393.

Nath A., Li I., Roberts L.R. and Chan C. (2015). Elevated free fatty acid uptake via CD36 promotes epithelial-mesenchymal transition in hepatocellular carcinoma. Sci. Rep. 5, 14752.

Palmieri D., Fitzgerald D., Shreeve S.M., Hua E., Bronder J.L., Weil R.J., Davis S., Stark A.M., Merino M.J., Kurek R., Mehdorn H.M., Davis G., Steinberg S.M., Meltzer P.S., Aldape K. and Steeg P.S. (2009). Analyses of resected human brain metastases of breast cancer reveal the association between up-regulation of hexokinase 2 and poor prognosis. Mol. Cancer Res. 7, 1438-1445.

Park S.Y., Kim H.S., Kim N.H., Ji S., Cha S.Y., Kang J.G., Ota I., Shimada K., Konishi N., Nam H.W., Hong S.W., Yang W.H., Roth J., Yook J.I. and Cho J.W. (2010). Snail1 is stabilized by O-GlcNAc modification in hyperglycaemic condition. EMBO J. 29, 3787-3796.

Pate K.T., Stringari C., Sprowl-Tanio S., Wang K., TeSlaa T., Hoverter N.P., McQuade M.M., Garner C., Digman M.A., Teitell M.A., Edwards R.A., Gratton E. and Waterman M.L. (2014). Wnt signaling directs a metabolic program of glycolysis and angiogenesis in colon cancer. EMBO J. 33, 1454-1473.

Pavlova N.N. and Thompson C.B. (2016). The emerging hallmarks of cancer metabolism. Cell. Metab. 23, 27-47.

Penny H.L., Sieow J.L., Adriani G., Yeap W.H., See Chi Ee P., San Luis B., Lee B., Lee T., Mak S.Y., Ho Y.S., Lam K.P., Ong C.K., Huang R.Y., Ginhoux F., Rotzschke O., Kamm R.D. and Wong S.C. (2016). Warburg metabolism in tumor-conditioned macrophages promotes metastasis in human pancreatic ductal adenocarcinoma. Oncoimmunology 5, e1191731.

Peppicelli S., Bianchini F. and Calorini L. (2014a). Extracellular acidity, a "reappreciated" trait of tumor environment driving malignancy: perspectives in diagnosis and therapy. Cancer Metastasis Rev. 33, 823-832.

Peppicelli S., Bianchini F., Torre E. and Calorini L. (2014b). Contribution of acidic melanoma cells undergoing epithelial-to-mesenchymal

transition to aggressiveness of non-acidic melanoma cells. Clin. Exp. Metastasis 31, 423-433.

Sajnani K., Islam F., Smith R.A., Gopalan V. and Lam A.K. (2017). Genetic alterations in Krebs cycle and its impact on cancer pathogenesis. Biochimie 135, 164-172.

Sánchez-Martínez R., Cruz-Gil S., Gómez de Cedrón M., Álvarez-Fernández M., Vargas T., Molina S., García B., Herranz J., Moreno-Rubio J., Reglero G., Pérez-Moreno M., Feliu J., Malumbres M. and Ramírez de Molina A. (2015). A link between lipid metabolism and epithelial-mesenchymal transition provides a target for colon cancer therapy. Oncotarget 6, 38719-38736.

Sansom O.J., Meniel V.S., Muncan V., Phesse T.J., Wilkins J.A., Reed K.R., Vass J.K., Athineos D., Clevers H. and Clarke A.R. (2007). Myc deletion rescues Apc deficiency in the small intestine. Nature 446, 676-679.

Sciacovelli M. and Frezza C. (2017). Metabolic reprogramming and epithelial-to-mesenchymal transition in cancer. FEBS J. 284, 3132-3144.

Seliger C., Leukel P., Moeckel S., Jachnik B., Lottaz C., Kreutz M., Brawanski A., Proescholdt M., Bogdahn U., Bosserhoff A.K., Vollmann-Zwerenz A. and Hau P. (2013). Lactate-modulated induction of THBS-1 activates transforming growth factor (TGF)-beta2 and migration of glioma cells *in vitro*. PLoS One 8, e78935.

Semenza G. (2013). HIF-1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations. J Clin Invest. 123, 3664-71.

Shen L., Qu X., Ma Y., Zheng J., Chu D., Liu B., Li X., Wang M., Xu C., Liu N., Yao L. and Zhang J. (2014). Tumor suppressor NDRG2 tips the balance of oncogenic TGF-β via EMT inhibition in colorectal cancer. Oncogenesis 3, e86.

Sheng S.L., Liu J.J., Dai Y.H., Sun X.G., Xiong X.P. and Huang G. (2012). Knockdown of lactate dehydrogenase A suppresses tumor growth and metastasis of human hepatocellular carcinoma. FEBS J. 279, 3898-3910.

Shrestha N., Chand L., Han M.K., Lee S.O., Kim C.Y. and Jeong Y.J. (2016). Glutamine inhibits CCl_4 induced liver fibrosis in mice and TGF-β1 mediated epithelial-mesenchymal transition in mouse hepatocytes. Food Chem. Toxicol. 93, 129-137.

Suzuki A., Maeda T., Baba Y., Shimamura K. and Kato Y. (2014). Acidic extracellular pH promotes epithelial mesenchymal transition in Lewis lung carcinoma model. Cancer Cell. Int. 14, 129.

Tamura T., Ichikawa T., Nakahata S., Kondo Y., Tagawa Y., Yamamoto K., Nagai K., Baba T., Yamaguchi R., Futakuchi M., Yamashita Y. and Morishita K. (2017). Loss of NDRG2 expression confers oral squamous cell carcinoma with enhanced metastatic potential. Cancer Res. 77, 2363-2374.

Tao Y., Guo Y., Liu W., Zhang J., Li X., Shen L., Ru Y., Xue Y., Zheng J., Liu X., Zhang J. and Yao L. (2013). AKT inhibitor suppresses hyperthermia-induced NDRG2 phosphorylation in gastric cancer cells. Braz. J. Med. Biol. Res. 46, 394-404.

Wang H., Wang W., Wang X., Cai K., Wu H., Ju Q., Huang Z. and Gao X. (2012). Reduced N-Myc downstream-regulated gene 2 expression is associated with CD24 upregulation and poor prognosis in patients with lung adenocarcinoma. Med. Oncol. 29, 3162-3168.

Wang J., Yin D., Xie C., Zheng T., Liang Y., Hong X., Lu Z., Song X., Song R., Yang H., Sun B., Bhatta N., Meng X., Pan S., Jiang H. and Liu L. (2014). The iron chelator Dp44mT inhibits hepatocellular carcinoma metastasis via N-Myc downstream-regulated gene 2 (NDRG2)/gp130/STAT3 pathway. Oncotarget 5, 8478-8491.

Warburg O. (1956). On the origin of cancer cells. Science 123, 309-214.

Ward P.S. and Thompson C.B. (2012). Metabolic reprogramming: a cancer hallmark even warburg did not anticipate. Cancer Cell. 21, 297-308.

Wick W., Naumann U. and Weller M. (2006). Transforming growth factor-beta: a molecular target for the future therapy of glioblastoma. Curr. Pharm. Des. 12, 341-349.

Xu Q., Zhang Q., Ishida Y., Hajjar S., Tang X., Shi H. and Dang C.V. (2017). EGF induces epithelial-mesenchymal transition and cancer stem-like cell properties in human oral cancer cells via promoting Warburg effect. Oncotarget 8, 9557-9571.

Xu X., Li J., Sun X., Guo Y., Chu D., Wei L., Li X., Yang G., Liu X., Yao L., Zhang J. and Shen L. (2015). Tumor suppressor NDRG2 inhibits glycolysis and glutaminolysis in colorectal cancer cells by repressing c-Myc expression. Oncotarget 6, 26161-26176.

Xu Y., Huang J., Xin W., Chen L., Zhao X., Lv Z., Liu Y. and Wan Q. (2014). Lipid accumulation is ahead of epithelial-to-mesenchymal transition and therapeutic intervention by acetyl-CoA carboxylase 2 silence in diabetic nephropathy. Metabolism 2014, 63, 716-726.

Yang P., Li Z., Fu R., Wu H. and Li Z. (2014). Pyruvate kinase M2 facilitates colon cancer cell migration via the modulation of STAT3 signalling. Cell. Signal. 26, 1853-1862.

Ye X. and Weinberg R.A. (2015). Epithelial-mesenchymal plasticity: A central regulator of cancer progression. Trends Cell. Biol. 25, 675-686.

Yu C., Wu G., Li R., Gao L., Yang F., Zhao Y., Zhang J., Zhang R., Zhang J., Yao L., Yuan J. and Li X. (2015). NDRG2 acts as a negative regulator downstream of androgen receptor and inhibits the growth of androgen-dependent and castration-resistant prostate cancer. Cancer Biol. Ther. 16, 287-296.

Zuo J., Wen J., Lei M., Wen M., Li S., Lv X., Luo Z. and Wen G. (2016). Hypoxia promotes the invasion and metastasis of laryngeal cancer cells via EMT. Med. Oncol. 33, 15.

Zhang M., Liu X., Wang Q., Ru Y., Xiong X., Wu K., Yao L. and Li X. (2017). NDRD2 acts as a PERK co-factor facilitate PERK branch and ERS-induced cell death. FEBS Lett. 26, 10.

Accepted December 29, 2017