

A New Insight into the Effect of Heme Oxygenase-1 in Progress of Malignant Tumors

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Received: September 14, 2012 Revised: October 19, 2012

Accepted: October 20, 2012

Published online: January 21, 2013

ABSTRACT

Heme Oxygenase-1 (HO-1) is a rate-limiting enzyme which catalyzes the oxidation of heme to biologically active products: ferrous iron, carbon monoxide (CO) and biliverdin. The biliverdin converts to bilirubin by biliverdin reductase. There are two isoforms of heme oxygenase. HO-1 is the highly inducible enzyme by heme and various other stimuli including oxidative stress. HO-2 is the constitutively expressed isoform. Since it had been found in 1986, a large number of studies have shown that HO-1 participated in various oxidative stress and inflammatory responses. The studies proved that it has anti-inflammatory, anti-apoptotic and cell protective effect. In recent years, studies have shown that HO-1 was highly expressed in a variety of malignant tumors and participated in anti-apoptotic in tumors. Also, it plays a regulatory role in tumor angiogenesis and metastasis. This article is not exhaustive but seeks to summarize what is known about the function of HO-1 in the development of malignant tumors.

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Key words: Heme Oxygenase-1; Tumor; Anti-apoptotic; Angiogenesis and metastasis

Bai FH, Qi NN, Wu CX, Nie YZ. A New Insight into the Effect of

Heme Oxygenase-1 in Progress of Malignant Tumors. *Journal of Gastroenterology and Hepatology Research* 2013; 2(1): 353-358
Available from: URL: <http://www.ghrnet.org/index./joghr/>

INTRODUCTION

HO is a rate-limiting enzyme in the formation of bilirubin. It catalyzes the rate-limiting step in heme degradation that results in the formation of biliverdin, which is subsequently converted to bilirubin by biliverdin reductase. During the degradation, Porphyrin release ferrous iron and carbon monoxide (CO). Heme oxygenase (HO) made its debut onto the scientific stage in 1964 when Wise and coworkers first demonstrated the degradation of heme to biliverdin in vitro^[1]. Further investigations by Tenhunen and colleagues have confirmed this finding^[2,3]. They identified HO as the enzyme responsible for the catabolism of heme and went on to characterize the enzyme and its cellular localization. In the late 1980s, an inducible form of HO called heme oxygenase-1(HO-1) was discovered^[4]. HO-1 proved to be identical to heat shock protein 32^[5]. This sparked new interest in HO as a stressresponsive protein. In 1985, heme oxygenase cDNA was cloned from the rat spleen using a specific antibody as a probe^[6]. Subsequently, a second form of heme oxygenase was discovered in the rat testis^[7,8]. The newly discovered enzyme was named heme oxygenase-2^[9]. In fact, HO-1 expression is induced by heme-tissue injury and other non-heme such as hydrogen peroxide, ultraviolet irradiation, cadmium, endotoxin, or nitric oxide. Recently, a redox-sensitive transcription factor, NF-E2-related factor-2 (Nrf2), was found to mediate the expression of many antioxidant protein such as HO-1^[10]. Yu Shan Wei and coworkers identified a peptidoglycan-like fraction, an extract from *G.tsugae*, that can act as a HO-1 protein expression enhancer in BAECs and they also linked its activation mechanism to Nrf2 dependent pathways^[10]. Previous studies have shown that HO-1 most likely has the role of anti-inflammatory and anti-oxidation. But in recent years, an increasing number of reports has argued that HO-1 has a regulatory role in malignant tumor. Compared with surrounding normal tissues, HO-1 expression is in the increasing trend of tumors. Otherwise, HO-1 is related to stress tolerance and apoptosis of tumor cells. HO-1 also has been recognized as a catalytic enzyme which stimulates tumor angiogenesis.

SEXPRESSION OF HO-1 IN TUMORS

High expression of the inducible isoform of HO-1 is well known in various solid tumors of humans and experimental animal models. HO-1 was thus considered to be a key molecule for tumors against the attack of host chemotherapy and radiotherapy by protecting tumor cells from oxidative insults^[11]. It has been found that several tumors, including renal cell carcinoma^[12] and prostate tumors^[13] in human, express a high level of HO-1. Lei Shi and coworkers^[11] suggested that expression of HO-1 in nasopharyngeal carcinoma is associated with the sensitivity to radiotherapy because high expression of HO-1 induces to resistance to radiotherapy. P Sacca and coworkers^[14] demonstrated that HO-1 expression and nuclear localization can define a new subgroup of prostate cancer primary tumors. Also the modulation of HO-1 expression and its nuclear translocation could represent new avenues for therapy. A recent study has show that HO-1 expression was significantly higher in Oral Squamous Cell Carcinoma specimens^[15]. They suggest that HO-1 expression is significantly up-regulated in Oral Squamous Cell Carcinoma from areca quid chewers. And arecoline may be responsible for enhanced HO-1 expression *in vivo*. To investigate the relationship between HO-1 and nasopharyngeal carcinomas, Jun Fang and coworkers^[11] have analyzed thirty-two nasopharyngeal carcinomas semiquantitatively by RT-PCR. They first demonstrated the expression of HO-1 in nasopharyngeal carcinomas, and more important their findings strongly suggest the potential of HO-1 as an useful index in identifying patients with well response to radiotherapy. Further datas indicated a new therapeutic for nasopharyngeal carcinoma by inhibitory HO-1 activity. The high expression of HO-1 has also been detected in various neoplastic cells, such as human adenocarcinoma, hepatoma, glioblastoma, squamous carcinoma, prostatic cancers, Kaposi sarcoma, and melanoma^[11,16,17,18]. More importantly, the expression of HO-1 in tumors can be potently induced in response to radio-, chemo-, or photodynamic therapies^[19,20,21].

THE ROLE OF HO-1 IN CELLS CYCLE

There are many studies have demonstrated that HO-1 play an important role in human pathophysiological pathway, but how HO system may regulate cell cycle and cell proliferation still need our further study. Giovanni Li Volit^[22] and coworkers used both pharmacologic agonists and antagonists for HO-1 had got different results in EC (endothelial cell) and SMC (smooth muscle cell). In ECs, the activation of HO-1 decreased G1 phase, but increased S and G2/M phase, inhibition of HO-1, increased G1 phase but decreased S and G2/M phase. The opposite effects were obtain in SMC. This result demonstrated that HO-1 regulates the cell cycle in cell-specific manner. The similar result was also demonstrated by Eun Mi Jean *et al*^[23]. When they studied the function of HO-1 in Hypertensive rats, they found that HO-1 could inhibit the proliferation of VSMC (vascular smooth muscle cell), and this effect could restore by SnPPiX. The main mechanism was that HO-1 could influence VSMC cycle. Treatment cells with hemin down-regulated the expression of cyclin D and upregulated p21 then inhibited the cell-cycle progression from the G1 phase to the S phase. Paola and coworkers^[24] used LLC-PK1 cells to examine the effect of overexpression of HO-1 on cell growth, expression of p21, and susceptibility to apoptosis. LLC-PK1 cells are stable overexpression of HO-1. At last the overexpression cells demonstrated cellular hypertrophy, hyperplastic growth were inhibited, and growth cycle arrested in the G0/G1 phase and resistance to apoptosis which was induced by TNF- α , cycloheximide,

staurosporine, or serum deprivation, p21 protein were also up-regulated. They argued that HO-1 is a potent stimulus to p21 and though p21 pathway regulate of the cell cycle. Some researchers^[25] found that p53 could promote cellular survival and this function is related with the expression of HO-1. P53 can be activated by cellular injuries and lead to cell-cycle arrest or apoptosis. But the recent study have found that p53 can also protect the cells survive. They also found that both mouse and human cells lacking p53 become sensitive to H₂O₂-induced cell death. In p53-proficient cells, HO-1 could directly be induced by H₂O₂. In p53-deficient cells, the inhibition of HO-1 could promote the cells apoptosis which were induced by H₂O₂, and this function can be restored by antioxidant treatment and bilirubin, bilirubin was produced by HO-1. However, the influential factors of cell cycle are multifactorial and cannot be defined by a single-gene, some researchers try to get some network about how HO-1 regulate cell cycle and proliferation. Huai-Wei Zeng and coworkers^[26] used a cancer signal pathway-specific array to study molecules which were involved in oncogenesis. The HT1080 fibrosarcoma were exposed in Methylselenol. Used real-time PCR, compared with control, they found that the expression of CDKN1C, HO-1, platelet/endothelial cell adhesion molecule and PPAR γ genes were up-regulated, Bcl-2-related protein A1, hedgehog interacting protein, and p53 target zinc finger protein genes were downregulated, and extracellular-regulated kinase 1/2 (ERK1/2) signaling and cellular myelocytomatosis oncogene (c-Myc) expression were also inhibited. In this study, they argued that CDKN1C, HO-1, platelet/endothelial cell adhesion molecule, and PPAR γ genes were related to the regulation of cell cycle and apoptosis. The regulation of those genes is likely to play a role in G1 cell cycle arrest and apoptosis. But the exact mechanisms of those gens how to effect the cell cycle and apoptosis still need to be further study. Abraham NG and coworkers^[27] used cDNA microarray technology to analyze genes which were regulated by HO-1 in the cell cycle. They used a retroviral vector encoded with human HO-1 gene or an empty vector to transfer human endothelial cells. At last, they found that in overexpression of human HO-1 cells some genes expression were changed. Cyclin E and D were upregulated, and cyclin-dependent kinase inhibitors p21 and p27, cyclin-dependent kinases 2, 5, and 6, and monocyte chemoattractant protein-1 were downregulate and those gens were associated with cell cycle progression; growth factors, including vascular endothelial growth factor (VEGF) and vascular endothelial growth factor receptor 1 (VEGFR1), endothelial growth factor (EGF) and hepatic-derived growth factor (HDGF) were all. They argued that these gens are all related to human HO-1 signaling involved in cell growth. Although, above studies have revealed some mechanisms about how HO-1 may regulate cell cycle and cell proliferation, the exact mechanism still need our further study.

THE ROLE OF HO-1 IN TUMOR GROWTH

HO-1 also affect tumor growth in the cancer cells. Lee YM and coworkers explored the effects of isoliquiritigenin 2'-methyl ether(ILME) that inhibited the growth of oral cancer cells in a time and dose-dependent manner through up-regulating HO-1 expression^[28]. Gueron G and partners^[29] studied the effect of genetic and pharmacologic disruption of HO-1 in the growth, invasion, and migration in androgen-sensitive (MDA PCa2b and LNCaP) and androgen-insensitive (PC3) PCa cell lines. Their results show that HO-1 levels are markedly decreased in PC3 compared with MDA PCa2b and LNCaP. Further more, overexpression of HO-1 in PC3 resulted in markedly reduced cell proliferation and migration. Zinc protoporphyrin IX is an endogenous heme analogue that inhibits heme

oxygenase activity. Fernando AP and coworkers have demonstrated that tumor cell proliferation was inhibited by Znpp^[30]. So we can assume that Znpp could inhibit the proliferation of tumor cells by inhibiting the activity of heme oxygenase-1. It indicated that HO-1 could promote the proliferation of tumor cells indirectly. But this conclusion was contrary to the idea that HO-1 could inhibit the growth of tumor cells which was mentioned earlier. So it is not one-sided view that heme-oxygenase-1 is to promote the growth or the inhibition of tumor cells. Gleixner KV's experiments showed that Hsp32 (HO-1) is expressed in most solid tumors and hematopoietic neoplasms and may be employed as a new therapeutic target as evidenced by experiments using specific siRNA and a Hsp32 targeting drug. SMA-Znpp was found to inhibit the proliferation of neoplastic cell with values ranging between 1 and 50 micro M. Study also have shown that siRNA-mediated knockdown of HO-1 could suppress the growth of bladder cancer cells over-expressing HO-1^[31].

In summary, it must be kept in mind that HO-1 does not always increase growth, it also has the effect of anti-proliferation.

HO-1 AND TUMOR APOPTOSIS

Expression of HO-1 plays a critical role in cytoprotection. There have been large number of studies that explore its effect of regulating anti-apoptotic in cells. A study suggests that HO-1 protects endothelial cells (EC) from undergoing apoptosis is mimicked by CO, which is generated via the catabolism of heme by HO-1^[32]. It depends on the activation of the p38 MAPK signal transduction pathway. The anti-apoptotic effect of CO in EC was abrogated when activation of the p38 α and p38 β MAPKs was inhibited. In cancer, HO-1 has been described as a protumoral molecule because of its antiapoptotic effects in colon cancer and liver cancer of murine models. Stuart. A Rushworth and coworkers^[33] study showed that the cytoprotective gene HO-1 was induced in AML-derived cell lines but not primary white blood cells under NF-KB-inhibited TNF-treated conditions. Moreover, induction of HO-1 protected AML-derived cells from undergoing cell death. In contrast, primary cells underwent apoptosis and did not induce HO-1 expression when treated with TNF in conjunction with NF-KB inhibition. Therefore, they suggested that TNF, in combination with NF-KB inhibition, generated ROS production in apoptosis-resistant leukemia cells, and this induced activation of the transcription factor Nrf2 to upregulate HO-1 expression, which subsequently provides cellular resistance to TNF-mediated apoptosis. Also HO-1 is considered to play an important role as a survival molecule in CML cells^[34] as overexpression of HO-1 was found to inhibit apoptosis induced by the BCR/ABL tyrosine kinase inhibitor imatinib (ST1517). Karolien V. Gleixner and coworkers^[34] confirmed the functional role of HO-1 as a survival-related molecule in CML cells by using 3 different HO-1-specific siRNAs. In their experiments, they were able to show that the siRNA-induced knockdown of HO-1 is associated with growth inhibition and induction of apoptosis in K562 cell. These data provide solid evidence for the functional role of HO-1 as an important survival factor in CML cells.

Aside from CO, bilirubin and biliverdin were indicated as antiapoptotic mediators in colon cancer cells^[35]. Interestingly, the induction of HO-1 is not always sufficient to protect the cells. As demonstrated in breast carcinoma and B-lymphoblasts, HO-1 did not protect the cells from chemotherapy-induced apoptosis^[36].

HO-1 AND TUMOR ANGIOGENESIS

The role of HO-1 in angiogenesis is complex, and remains to be

fully understood and presence dates show that HO-1 may influence the angiogenic process at different levels. HO-1 as an inducers of angiogenesis may by stimulating the expression of VEGF. HO-1-deficient endothelial cells produced less vascular endothelial growth factor (VEGF) and their response to exogenous VEGF and basic fibroblast growth factor (bFGF) was weaker than the wild-type counterparts^[37]. The results of experiments performed *in vitro* have been confirmed *in vivo* in the rat ischemic hindlimb model, in which HO-1 gene transfer increased VEGF synthesis, facilitated angiogenesis, and improved the blood flow in the ischemic muscles^[38]. Li Volti G and coworkers used pharmacologic agonists and antagonists for HO-1 had revealed that HO-1 increasing EC cycle progression while inhibiting that of VSMCs^[39]. Inhibition of HO-1 expression, cell-cycle progression and EC proliferation, and capillary formation were all inhibited^[40]. These responses were significantly reversed by CO^[41]. Li Volti G and coworkers^[40] used HO-1-deficient endothelium cells to determine whether supplement with CO or bilirubin would regulate cell proliferation and angiogenesis. They found that down-regulated HO-1 expression could significantly decrease the level of VEGF and this effect could be restored by CO but not bilirubin. They also found that HO-1 deficiency could increase the expression of p21, p27 and PGE2. This study suggested that HO-1-driven CO mediate angiogenesis. SDF-1 (stromal cell-derived factor 1) is widely distributed and induced by a range of pathophysiological event such as ischemia and inflammation^[42]. Recently, Jessy and partners^[43] demonstrated that SDF-1 activated HO-1-expression in human and mouse aortic endothelial cells (MAECs) and mouse EPCs. The inhibition of HO-1 expression resulted in the loss of SDF-1-mediated endothelial tube formation and aortic rings activation. This effect could be restored by CO but not bilirubin. They also demonstrated that SDF-1-mediated occurs by a protein kinase C (PKC) ζ -dependent and VEGF-independent mechanism. HIF-1 α (hypoxia-inducible factor 1 α) a nuclear transcription factor induced by hypoxia regulate the response of a variety of genes to hypoxic stimulus. HIF-1 α is important in keeping stabilization of oxygen, cell energy metabolism, the vasification of tumor, cell proliferation and apoptosis. A study demonstrated that Carbon Monoxide(CO) promotes VEGF expression by increasing HIF-1 α protein level via two distinct mechanisms, translational activation and stabilization of HIF-1 α protein^[44]. Most of results demonstrated that HO-1 is an important factors for angiogenesis, and in the HO-1-induced angiogenesis pathway CO apparently play an important role, but the exact mechanism of HO-1-induced angiogenesis still need our further study.

The solid tumors growth, invasion and metastasis are all need angiogenesis and the above studies clearly showed that heme oxygenase-1 (HO-1) plays an important role in angiogenesis. Maybe the HO-1 also has an important role in tumors. A study revealed that HO-1 stimulates angiogenesis of pancreatic carcinoma in severe combined immune deficient mice^[45]. Over-expression of human HO-1 after its retroviral transfer into pancreatic cancer cells did not interfere with tumor growth *in vitro*. While *in vivo*, the development of tumors was accelerated upon transfection with hHO-1. On the other hand, inhibition of HO activity by stannous mesoporphyrin was able transiently delay tumor growth in a dose dependent manner. Tumor angiogenesis was markedly increased in Panc-1/hHO-1 compared to mock transfected and wild type. Another study thought that macrophages are key participants in angiogenesis^[46]. They first investigated whether macrophage infiltration is associated with angiogenesis and malignant histological appearance in human brain tumors. More importantly, HO-1, the rate-limiting enzyme in

heme catabolism, was also associated with activated macrophages. Immunostaining of macrophages and small vessels in resected glioma specimens indicated that numerous infiltrating macrophages and small vessel density were higher in glioblastomas than in astrocytomas or anaplastic astrocytomas. Macrophage infiltration was closely correlated with vascular density in human gliomas. A new report suggested that copper is required for the proliferation of endothelial cells and copper-lowering therapy reduces tumor growth in animal models^[33]. ATN-224, a novel copper chelator, potently inhibits the activity of the copper-dependent enzyme superoxide dismutase 1 (SOD1) in endothelial cells. Sarah A. Lowndes and coworkers^[47] performed microarray analysis of gene expression in endothelial cells exposed to ATN-224 which revealed up-regulation of stress response genes including HO-1 and differential regulation of several genes previously implicated in angiogenesis. Their data lead to the confirmation of SOD1 as an early target of ATN-224. Signaling pathways involved in free radical signaling and HO-1 up-regulation were then investigated in order to confirm the link between SOD1 and HO-1. The genes that were differentially regulated by ATN-224 at 24 and 48 h and previously known to be involved in angiogenesis were confirmed using quantitative real time PCR. That means HO-1 could regulate the angiogenesis in some degree. Dan Meng and coworkers reported the mechanism of arsenic promotes angiogenesis *in vitro* via a heme oxygenase-1-dependent manner^[48]. Arsenic has been shown to stimulate angiogenesis and vascular remodeling *in vivo*. But the exact molecular mechanisms accounting for arsenic-induced angiogenesis are not clear. Their study investigates the role of heme oxygenase-1 in sodium arsenite-mediated angiogenesis *in vitro*. They reported that low concentrations of arsenic (0.1-1 μ M) stimulated cell migration and vascular tube formation in human microvascular endothelial cells (HMVEC). Arsenite induced HO-1 mRNA and protein expression. Knock down of HO-1 expression decreased arsenite-induced VEGF expression, cell migration and tube formation. It was shown that arsenite promoted dissociation of Bach1 (a transcriptional repressor) from the HO-1 enhancers and increased Nrf2 binding to these elements. Site directed mutagenesis assay identified that Bach 1 cysteine residues 557 and 574 were essential for the induction of HO-1 gene in response to arsenite. Although, the exact function of HO-1 in tumors angiogenesis remains to further study, but more attention should be paid on the role of HO-1 in tumor angiogenesis.

HO-1 AND TUMOR METASTASIS

As above, we talk something about HO-1 in tumor angiogenesis. Angiogenesis is critical not only for tumor growth but also for tumor metastasis. The tumor metastasis is a highly complex and multistep process that requires a tumor cell to modulate its ability to adhere, degrade the surrounding extra cellular matrix, migrate, proliferate at a secondary site, and stimulate angiogenesis. The progression of a tumor from benign and delimited growth to invasive and metastatic growth is the major cause of poor clinical outcome in cancer patients^[49].

In the tumor cells *in vitro*, Halina Was and coworkers^[50] investigated the role of HO-1 in B16 (F10), S91, and Sk-mel188 melanoma cells. Tumors over-expressing HO-1 displayed augmented vascularization and stronger production of vascular endothelial growth factor. Finally, B16-HO-1 cells (cells stably transfected with HO-1 cDNA) injected intravenously formed more metastases in lungs. Likewise, increased occurrence of lung metastases has been described in the rats bearing pancreatic cancer implants over-expressing HO-1^[51], suggesting that pro-metastatic action of HO-1 is a more general event. Data obtained from microarrays suggested

that formation of metastases by B16-HO-1 is associated with an increased level of T β 4. This small protein is known to be up-regulated in metastatic nodules and its expression correlates with metastatic potential of tumors mostly by activating cell migration and stimulating angiogenesis. Another experimental model report T β 4 overexpressing B16 (F10). Melanoma cells injected intravenously to mice produced almost fivefold more metastatic lung nodules than the wild-type cells^[52]. Hyaluronidase HYAL1 another pro-metastatic gene is also up-regulated in B16-HO-1, which degrades hyaluronic acid, involved in regulation of cell adhesion and migration to small pro-angiogenic fragments. Over-expression of HYAL1 in bladder cancer cells increases their invasiveness^[53].

A (GT)_n dinucleotide repeat in 5'-flanking region of the human HO-1 gene shows length polymorphism. There are three classes: class S (<27 GT), class M (27–32 GT), and class L (\geq 33 GT) alleles^[54]. In polymorphic analysis, longer (GT)_n repeats in the HO-1 gene promoter are associated with increased risk of various cancers such as esophageal squamous cell carcinoma^[55], urothelial carcinomas^[56], oral squamous cell carcinoma^[57], lung adenocarcinoma^[54]. Heme Oxygenase-1 gene promoter polymorphism is not only effect tumors progression but also have relation with the tumor invasion. A finding found that HO-1 gene promoter polymorphism associated with risk of gastric adenocarcinoma and lymphovascular tumor invasion^[58]. They used PCR-based genotyping and DNA sequencing to examine the genotypic frequencies of (GT)_n repeats in 183 gastric cancer patients and 250 control subjects. The result suggest that the long (GT)_n repeat of HO-1 gene promoter was associated with a high frequency of gastric adenocarcinoma and the medium (GT)_n repeat might possess protective effect against gastric adenocarcinoma with a lower frequency of lymphovascular invasion in tumors. A new recent study investigated the role of HO-1 in the metastatic potential of human breast cancer cells^[59]. They used 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂(15d-PGJ₂) to treat MCF-7 and MDA-MB-231 cells which increased the expression of HO-1 that preceded the induction of matrix metalloproteinases (MMPs). They also found that over-expression of HO-1 in the MCF-7 cells caused the induction of MMP-1 expression. In conclusion, 15-d-PGJ₂ up-regulates MMP-1 expression via induction of HO-1 and subsequent production of iron capable of generating ROS, which it may contribute to increased metastasis and invasiveness of human breast cancer cells.

Likewise, our previously work demonstrated that HO-1 participate in the regulation of gastric cancer peritoneal metastasis. ZnPPiX of HO-1 inhibitor may inhibit the mice gastric cancer peritoneal metastasis via inhibit the formation of tumor angiogenesis. Furthermore, we will continue to explore the exact mechanism of HO-1 in the gastric cancer peritoneal metastasis.

Summary

Recently, more and more studies have demonstrated that HO-1 may play an important role in tumor. However, the exact mechanism is still not very clear. Function studies have revealed that HO-1 was highly expressed in various solid tumors in human and experimental animal models. HO-1 also regulates the cell cycle in cancer cells. But it should be kept in mind that HO-1 does not always increase growth, it also has the effect of anti-proliferation. HO-1 is a kind of cytoprotective enzyme that plays an anti-apoptosis effect in tumor cells. Last but not least, HO-1 has also regulated the tumor angiogenesis and metastasis. We will attempt to explore the potential target of HO-1 in anti-tumor therapy. We hope that HO-1 inhibitors or its siRNA may exert anti-tumor therapy in some cancer patients. Of course, these are just our best wishes.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (NO. 81160308; NO.30860332 and NO.30670969).

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