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LRIG1, human EGFR inhibitor, reverses multidrug resistance through modulation of ABCB1 and ABCG2

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ABSTRACT

In our previous study, we have found that leucine-rich repeats and immunoglobulin-like domains 1(LRIG1) can improve the chemosensitivity in U251 cells whereas the role of LRIG1 in multidrug resistance (MDR) remains unknown. Here, we reported that LRIG1 can reverse MDR by inhibiting epidermal growth factor (EGF) receptor (EGFR) and secondary inhibiting ATP-binding cassette, sub-family B member 1(ABCB1) and ATP-binding cassette, sub-family G (WHITE), member 2 (ABCG2). Our data showed that the expression of LRIG1 was significantly higher in O6-methylguanine DNA methyltransferase (MGMT) Promoter Methylation positive glioblastoma tissues compared to MGMT Promoter Methylation negative glioblastoma tissues. In addition, we found that LRIG1 expression was significantly decreased in MDR cells U251/TMZ compared to U251cells. Our results demonstrated that over-expression of LRIG1 can reverse the MDR. The expression of ABCB1 and ABCG2 were markedly suppressed when LRIG1 was over-expressed, supporting the negative relationship between LRIG1 level and ABCB1 and ABCG2 level in human specimen. Furthermore, we found that LRIG1 downregulated ABCB1 and ABCG2 through suppressing EGFR expression. In case of EGFR knockdown, the effect of LRIG1 on regulating MDR, ABCB1 and ABCG2 was partially compromised. Our results, for the first time, showed that LRIG1 can reverse MDR in glioblastoma, by negatively regulating EGFR and secondary suppressing the levels of ABCB1 and ABCG2.

1. Introduction

Glioblastoma multiforme (GBM) is the most common and lethal type of primary brain tumor (Kotliarova and Fine, 2012). Even treated aggressively by surgery, radiation, and chemotherapy, the survival of patients with GBM still remains not more than 14 months (Nikaki et al., 2012). Since the introdution of temozolomide (TMZ), which has demonstrated promising activity against glioblastoma, as chemotherapeutic agent in clinic setting, chemotherapy has become more and more important. However, the clinical response to TMZ lasts only a few months and drug resistance subsequently

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develops in most cases (Spinelli et al., 2012), especially multidrug resistance (MDR), which has become the major the problem needed to be solved as soon as possible. Unfortunately, the mechanism of MDR was still unclear.

LRIG1 was an inhibitor of receptors tyrosine kinase, which had cDNA cloned and characterized in 1996 (Suzuki et al., 1996). The LRIG1 integral membrane protein has been demonstrated to regulate various oncogenic receptor tyrosine kinases, including EGFR, by cell-autonomous mechanisms (Yi et al., 2011). Previous studies have also showed that upregulation of LRIG1 suppresses malignant glioma cell growth by attenuating EGFR activity (Yi et al., 2011) and LRIG1 negatively regulates the oncogenic EGF receptor mutant EGFRvIII, which is the most common EGFR mutant observed in GBM (Stutz et al., 2008). Recently, the LRIG1 isoform has been linked to aggressive behaviors and drug resistance in esophageal carcinoma cell lines (Wu et al., 2012). Furthermore, our previous study identified LRIG1 as a possible candidate gene affecting the sensitivity of human glioblastoma to the TMZ (Liu et al., 2013). Recent studies found that octreotide enhances the sensitivity of the SKOV3/DDP ovarian cancer cell line to cisplatin chemotherapy in vitro by inhibiting EGFR (Shen et al., 2011), and more important, GW583340 and GW2974, two human EGFR and HER-2 inhibitors, reverse ABCG2- and ABCB1-mediated drug resistance (Sodani et al., 2012). Therefore, LRIG1 may reverse the MDR, but this still remains to be elucidated.

We hypothesized that LRIG1 is involved in the MDR of GBM and that can reverse MDR through suppressing the expression of EGFR and then suppressing ABCB1 and ABCG2. To test this hypothesis, we manipulated expression of LRIG1 in MGMT Promoter Methylation positive and negative patient samples, and examined whether LRIG1 can reverse MDR in MDR cells as well as the effect of LRIG1 on EGFR, ABCB1 and ABCG2. LRIG1 attracted our attention because previous studies had shown that the expression of LRIG proteins played an important role in the pathogenesis of astrocytic tumors (Yi et al., 2009). In this study, we used U251 and U87 cells as research models, which are the most frequent human primary brain tumors and represent the most malignant stage of GBM progression.

2. Results

2.1. *LRIG1 protein and mRNA level were upregulated in MGMT Promoter Methylation positive glioblastoma and downregulated in MDR cell line U251/TMZ cells*

To explore the relationship of LRIG1 expression and glioblastoma MDR, we assessed LRIG1 mRNA and protein expression in a series of primary human glioblastoma by real-time PCR and western blot (Fig. 1A and B). As shown in Fig. 1A and B, all 17 MGMT Promoter Methylation positive primary human glioblastoma and 17 MGMT Promoter Methylation negative primary human glioblastoma showed LRIG1 mRNA and protein expression. In addition, we found that patients with MGMT Promoter Methylation positive had significantly higher LRIG1 expression compared to the patients with MGMT Promoter Methylation negative.

2.2. *LRIG1 knockdown abrogated MDR in U251/TMZ cells*

To determine the role of LRIG1 in MDR development, we knocked down LRIG1 in U251 cells using siLRIG1. The downregulation of target protein was confirmed via western blot analysis (Fig. 2A). Article has reported that caspase 3 is activated in the apoptotic cell and has been widely used as readout for drug sensitivity (Yan et al., 2015). Our data showed that the expression of caspase 3 was increased (Fig. 2A) and the apoptosis rate of cells was significantly decreased when treated with TMZ (16 μg/ml) and etoposide (VP-16) (10 μg/ml) (Fig. 2E), suggesting that suppressing LRIG1 expression resulted in reversal of MDR. To solid this data, LRIG1 plasmid was also transfected into U251/TMZ cells which was established by us before (Liu et al., 2013). The upregulation of target protein was also confirmed via western blot analysis (Fig. 2B). In line with the results in U251 cells, our data showed that drug resistance to TMZ and VP-16 was abolished by upexpressed LRIG1 (Fig. 2B and F).

2.3. *LRIG1 inhibited ABCB1 and ABCG2*

In order to explore the underlying mechanisms of how LRIG1 reversed MDR, LRIG1 was over expressed in U251/TMZ cells and the expression levels of ABCB1, LRP, ABCG2 and MRP1, which was related to MDR (Liu et al., 2013), was determined by western blot and real-time PCR. Our data showed that LRIG1 overexpression did not result in change in levels of LRP and MRP1, but remarkably decreased ABCB1 and ABCG2 expression by more than 50% at both mRNA and protein level (there was a significant difference between expression levels of ABCB1 and ABCG2 between control and LRIGI overexpressing groups ($P<0.05$)) (Fig. 3A and B). In addition, we found that LRIG1 expression was significantly negatively correlated with ABCB1 (Pearson, $R^2=0.204$, $P=0.0037$) and ABCG2 (Pearson, $R^2=0.301$, $P=0.0004$) expression in human samples (Fig. 4C and D).

2.4. *LRIG1 suppressed EGFR level*

Previous studies have pointed out that EGFR inhibition affected ABCG2 expression in EGFR-positive MDCK BCRP cells via the PI3K/Akt signaling pathway (Pick and Wiese, 2012) and ABCB1 expression in acute lymphoblastic leukemia cells through MAPK/ERK pathway (Tomiyasu et al., 2013). We have found that LRIG1 can reverse MDR and repress ABCG2 and ABCB1 expressions in U251/TMZ cells. Therefore, we conducted experiments to test whether LRIG1 regulate ABCG2 and ABCB1 by inhibiting EGFR activity and secondary inhibition of PI3K/Akt signaling pathway and MAPK/ERK pathway in human MDR cells. First, we test the effect of LRIG1 on EGFR, p-AKT and p-ERK expression level. As expected, forced overexpression of LRIG1 in U251/TMZ cells significantly inhibited EGFR and p-AKT and p-ERK protein expression (Fig. 4A and B). To solid this data, the expression levels of LRIG1 and EGFR in human glioblastoma were analyzed by western blot. The results also showed that LRIG1 expression was significantly negatively correlated with EGFR levels