ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIII, N 3, 22 SECTIO DDD 2010

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Blockade of the endoplasmic reticulum stress sensor inositol requiring enzyme-1 changes the expression of cyclin and growth arrest-specific genes in glioma cells

Blokada enzymu-1 zależnego od inozytolu sensora stresu retikulum endoplazmatycznego zmienia ekspresję cyklin genów hamujących wzrost (GAS) w komórkach glejaka

INTRODUCTION

Astrocytes represent the most abundant cell type in the mammalian brain and play an important role in the maintenance and regeneration of neuronal functions. Ischemia have been shown to induce a set of complex intracellular signaling events known as the Unfolded Protein Response (UPR), which is mediated by inositol requiring enzyme-1 (IRE-1), to adapt for cell survival or, alternatively, to enter cell death programs through endoplasmic reticulum-associated machineries [2-5]. As such, it participates in the early cellular response to the accumulation of misfolded proteins in the lumen of the endoplasmic reticulum, occurring under both physiological and pathological situations. Two distinct catalytic domains of the dual enzyme IRE1, a serine/threonine kinase and an endoribonuclease contribute to IRE-1 signaling. IRE-1-associated kinase activity autophosphorylates and dimerizes IRE-1, leading to the activation of its ribonuclease domain, to the degradation of a specific subset of mRNA and to the initiation of the pre-XBP1 (X-box binding protein 1) mRNA splicing. Mature XBP1 mRNA encodes a transcription factor that stimulates the expression of UPR-specific genes [7, 10, 11, 14, 15]. Recently, single mutations in *IRE1*α gene were detected in human cancers and IRE1 was proposed as a major contributor to tumor (including glioblastoma) progression among protein kinases. IRE1 transduction pathway is linked to the neovascularization process, tumor growth and cellular death processes because a complete blockade of IRE1 activity had anti-tumor effects [1, 6, 8, 9, 12].

We studied effect of IRE-1-deficiency on the expression of different cyclin (A2, D3, E2 and G2) and cyclin-dependent kinase (CDK4 and CDK5) genes as well as growth arrest-specific genes GAS1 and GAS6 in glioma cell line U87 and modified glioma cells without IRE-1 kinase and ribonuclease activities.

MATERIAL AND METHODS

IRE-1 wild-type and knockout glioma cell line U87 were maintained in DMEM supplemented with 10% fetal bovine serum at 37°C in a 5% CO_2 incubator. RNA was extracted using Trizol reagent (Invitrogen, USA) [13]. Expression of cyclin A2, D3, E2, G2, CDK4 and CDK5 as well as growth arrest-specific genes GAS1 and GAS6 in glioma cell line U87 and its subline with IRE-1-deficiency was measured by quantitative polymerase chain reaction using „Stratagene Mx 3000P cycler" (USA) and SYBRGreen Mix (AB gene, Great Britain). QuaniTect Reverse Transcription Kit (QIAGEN, Germany) was used for cDNA synthesis. Polymerase chain reaction was performed in triplicate. The analysis of quantitative PCR was performed using a special computer program "Differential expression calculator" and statistical analysis – in Excel program.

RESULTS

As shown in Fig. 1 and 2, cyclin A2, D3 and G2 mRNA expression significantly increased (+39, +74 and +70 %, correspondingly) in IRE-1-deficient glioma cells as compared to control cell line. Both cyclin A2 and D3 mRNA expression in wild-type and IRE-1-deficient glioma cells is suppressed in hypoxic conditions. Much stronger (3 fold) induction of mRNA expression in IRE-1-deficient cells was shown for cyclin E2 (Fig. 2). Hypoxia also suppressed the expression of cyclin E2 mRNA both in wild-type and IRE-1-deficient glioma cells however, the expression of cyclin G2 mRNA was significantly induced (4 fold) under hypoxia in wild-type glioma cells and slightly less (3 fold) in cells with IRE-1-deficiency as compared to control cells (Fig. 2). Expression of both cyclin-dependent kinases CDK4 and CDK5 in IRE-1-deficient glioma cells did not change significantly as compared to control cells, but hypoxia is reduced expression of both kinases both in wild-type and IRE-1-deficient glioma cells (Fig. 3). We also showed that expression of both growth arrest-specific genes (GAS1 and GAS6) is hypoxia responsive and strongly enhanced in glioma cells without IRE-1 kinase and ribonuclease activities: in 7.3 and 6.7 fold, correspondingly (Fig. 4).

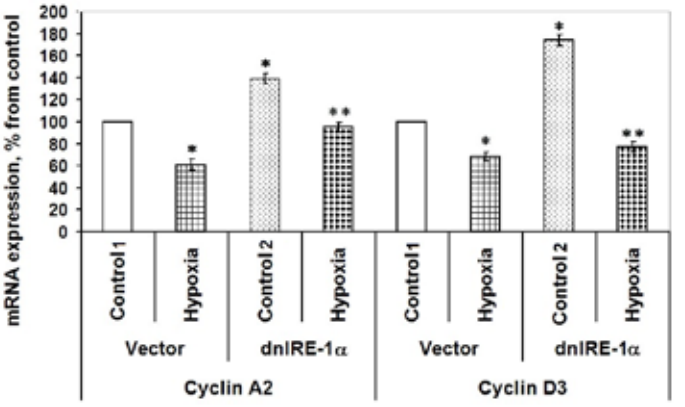

Fig.1. Expression of cyclin A2 and D3 mRNA in glioma cell line U87 and its subline with IRE-1-deficiency measured by quantitative polymerase chain reaction. Values of cyclin A2 and D3 mRNA expressions were normalized to β-actin mRNA expression. In Fig. 1-4: * - $P < 0.05$ as compared to control 1; ** - $P < 0.05$ as compared to control 2; $\boldsymbol{n} = 3$

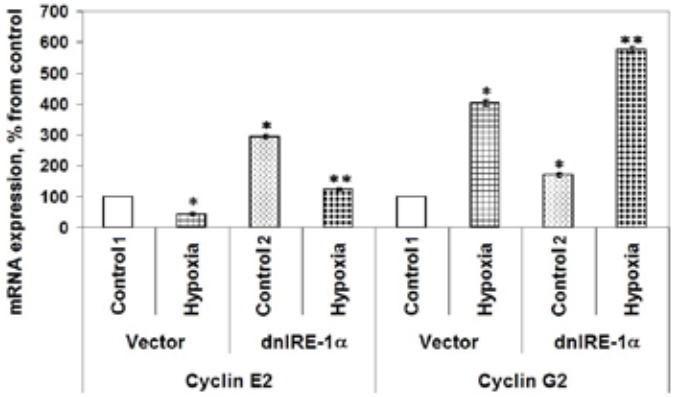

Fig. 2. Expression of cyclin E2 and G2 in glioma cell line U87 and its subline with IRE-1-deficiency measured by quantitative polymerase chain reaction. Values of cyclin E2 and G2 mRNA expressions were normalized to β-actin mRNA expression

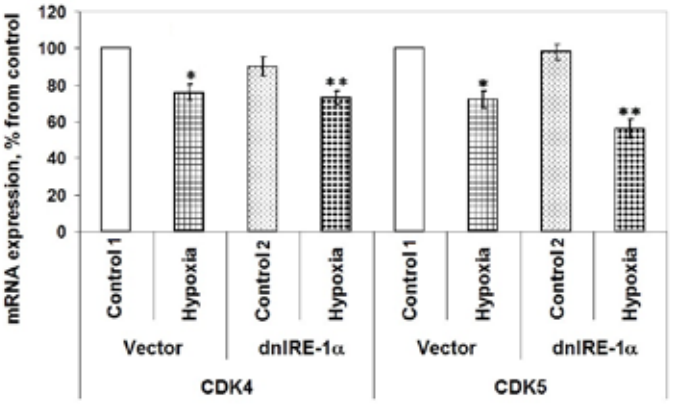

Fig. 3. Expression of cyclin-dependent kinase CDK4 and CDK5 in glioma cell line U87 and its subline with IRE-1-deficiency measured by quantitative polymerase chain reaction. Values of CDK4 and CDK5 mRNA expressions were normalized to β-actin mRNA expression

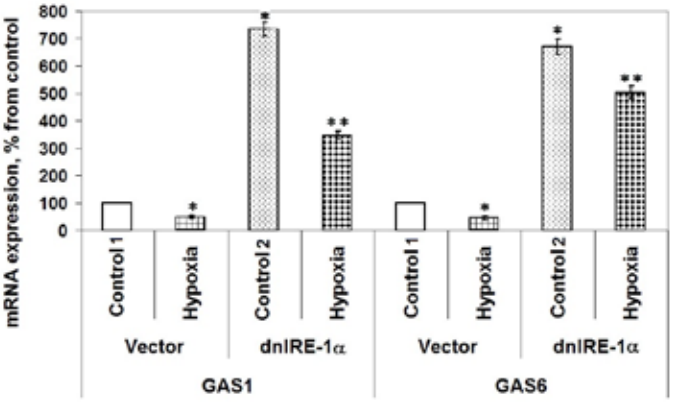

Fig. 4. Expression of growth arrest-specific genes GAS1 and GAS6 in glioma cell line U87 and its subline with IRE-1-deficiency measured by quantitative polymerase chain reaction. Values of cyclin GAS1 and GAS6 mRNA expressions were normalized to β-actin mRNA expression

DISCUSSION

Cyclin A2, D3, G2 and E2 as well as cyclin-dependent kinases CDK4 and CDK5 play a significant role in the cell cycle control and tumor growth [10, 15]. We observed induction of cyclin genes expression in IRE-1-deficient glioma cells which have suppressed cell proliferation and angiogenesis [6, 14]. Recently, IRE1 was proposed as a major contributor to tumor (including glioblastoma) progression among protein kinases because IRE1 transduction pathway is linked to the neovascularization process, tumor growth and cellular death processes [1, 10, 11, 15]. However, single mutations in *IRE1*α gene were detected in many human cancers. We also showed that a complete blockade of IRE1 activity significantly increased the expression of growth arrest-specific genes which correlate with anti-tumor effects of this IRE1 blockade.

CONCLUSIONS

Results of these investigations clearly demonstrated that the expression of different cyclin as well as growth arrest-specific genes in glioma cells is regulated by hypoxia and significantly depends from IRE-1 kinase and ribonuclease activities. Thus, cyclin A2, D3, E2 and G2 as well as GAS1 and GAS6 genes possibly participate in cell adaptive response to endoplasmic reticulum stress associated with accumulation of misfolded proteins in the endoplasmic reticulum.

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SUMMARY

We studied effect of inositol requiring enzyme-1 (IRE-1) deficiency on the expression of different cyclin and cyclin-dependent kinase (CDK) genes as well as growth arrest-specific (GAS) genes in glioma cells and modified glioma cells without IRE-1 kinase and ribonuclease activities. Cyclin A2, D3, E2 and G2 mRNA expression was significantly increased in IRE-1-deficient glioma cells as compared to control cell line. Blockade of IRE-1 activities significantly induced the expression of growth arrest-specific genes GAS1 and GAS6. Results of these investigations clearly demonstrated that the expression of growth arrest-specific genes and different cyclins in glioma cells significantly depends on IRE-1 kinase and ribonuclease activities.

Keywords: inositol requiring enzyme-1, gene expression, cyclin A2, D3, E2 and G2, cyclin-dependent kinase CDK4, growth arrest-specific genes GAS1 and GAS6, glioma cells

STRESZCZENIE

Zbadano wpływ niedoboru enzymu-1 zależnego od inozytolu (IRE-1) na ekspresję cyklin i genów kinazy zależnej od cyklin (CDK) jak też genów hamujących wzrost (GAS) w komórkach glejaka i modyfikowanych komórkach glejaka bez aktywności kinazy IRE-1 i rybonukleazy. Ekspresja cyklin A2, D3, E2 i G2 mRNA była istotnie wyższa w komórkach z niedoborem IRE-1 w porównaniu z kontrolną linią komórek. Zahamowanie aktywności IRE-1 w sposób istotny indukowało ekspresję genów hamujących wzrost GAS1 i GAS 6. Wyniki badań wskazują, iż ekspresja genów GAS oraz cyklin w komórkach glejaka zależy w głównej mierze od aktywności kinazy IRE-1 i rybonukleazy.

Słowa kluczowe: enzym-1 zależny od inozytolu, ekspresja genu, cykliny A2, D3, E2 i G2, kinaza cyklino-zależna CDK4, geny hamujące wzrost GAS1 i GAS6, komórki glejaka