Autophagy and GAPDH Increase in the Etiology of Acute Myeloid Leukemia With Nucleophosmin Mutation

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ABSTRACT

Resistance against apoptosis is a hallmark of cancer cells including acute myeloid leukemia (AML). It is reported that mutation in nucleophosmin, which causes its translocation from nucleolus into cytoplasm, was found in 30% of the primary AML patients. Previous results from our laboratory have shown that the mutant NPM (cNPM) could inhibit caspase-dependent cell death (CDCD) via inactivation of caspase-6 and -8. The purpose of this study is to investigate the relationship between overexpression of cNPM and caspase-independent cell death (CICD). Western blot and laser confocal microscopy were performed to assess the expression levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and Autophagy-Related 12 homolog (ATG12); and the extent of autophagic activities. Our data shows that green fluorescent protein (GFP)-tagged cNPM overexpressing human embryonic kidney (HEK) 293T cells manifest elevated autophagic activities. Furthermore, overexpression of mutant cNPM led to markedly high expression of GAPDH; without altering the expression of ATG12, a protein involved in autophagosome formation. Therefore, our data indicate that mutant cNPM may play a role in promoting autophagy through elevation of GAPDH expression level. This may play a role in leukemogenesis in AML patients with NPM mutation. The exact autophagy related genes involved in this phenomenon, however, need to be further investigated.

INTRODUCTION

Nucleophosmin (NPM) is an acidic and abundant nucleolar protein which translocates between the nucleus and cytoplasm (Yun et al., 2003). However, mutant nucleophosmin, cNPM was found in 30% of the primary Acute Myeloid Leukemia patients. Previous results from our laboratory have shown that cNPM could inactivate caspase-6 and caspase-8 which then inhibit caspase-dependent cell death (CDCD). It also suggests that cNPM could increase expression of glyceraldehydes-3-phosphate dehydrogenase (GAPDH) and Autophagy Related 12 homolog (ATG12), a protein involved in formation of autophagosomes. Previous studies have shown that without caspase activation, GADPH plays roles in preserving cell survival from caspase-independent cell death (CICD) by elevating glycolysis and enhancing autophagy, via increased expression of ATG12 (Colell et al., 2007). Thus, we hypothesized that cNPM may be involved

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in preventing cell death in AML. The purpose of this study is to investigate the relationship between overexpression of cNPM and CICD.

MATERIALS AND METHODS

Human embryonic kidney (HEK) 293T cells were transfected with pACGFP plasmids comprising gene constructs for green fluorescent protein (GFP), GFP-wild type NPM (GFP-wtNPM) or GFP cytosolic mutant NPM (GFP-cNPM), while for microscopy samples were also co-transfected with red fluorescent protein-tagged microtubule-associated protein 1 light chain 3 (RFP-LC3). These overexpressing cells were then pretreated with chloroquine for 1 hour followed by treatment with staurosporine (or DMSO as control) for 2 hours. The cells were harvested and lysed with total cell lysis buffer. The supernatant was then quantified using Bradford assay for protein concentration. Proteins were separated in polyacrylamide gels and electroblotted onto nitrocellulose membranes. Nitrocellulose membranes were incubated overnight with primary antibodies which were anti-LC3, -GADPH, -ATG12, and -β-actin. The membranes were then incubated with HRP-conjugated goat anti-mouse or anti-rabbit secondary antibodies. Protein bands were visualized with Pierce Biotechnology SuperSignal West Pico and Femto chemiluminescent substrates. For microscopy samples, the cover slips with cells were mounted onto glass slides with VECTASHIELD mounting medium containing DAPI. These samples were then viewed under laser scanning confocal microscope.

RESULTS

Overexpression of cNPM enhances autophagic activities

In this study, we hypothesized that overexpression of cNPM will promote autophagy, which may be involved in preserving blast cells survival in AML patients. Microtubule-associated protein 1A/1B-light chain 3 (LC3) serves as an indicator for autophagy as it is found present within autophagosome (Tanida et al, 2008). The comparison of LC3-II: LC3-I ratio between samples was used to examine the extent of autophagic activities. It was observed that GFP-cNPM overexpressing cells with drug treatments increase the LC3-II: LC3-I ratio. Furthermore, laser confocal microscopy showed that in cNPM overexpressing cells treated with chloroquine and staurosporine had the most number of autophagosome. Thus, these results illustrate that only cNPM could elevate the LC3-II: LC3-I ratio especially when the cells were challenged with staurosporine.

Overexpression of cNPM causes elevation of GADPH expression

Previous studies have shown that up-regulation of GAPDH expression indirectly elevating autophagic activities (Colell et al, 2007). Our data showed that regardless to drug treatment, GFP-cNPM overexpressing cells had markedly higher expression level of GADPH when compared to GFP and GFP-wtNPM overexpressing cells; and this may lead to elevation of autophagic activities as suggested by Colell et al (2007).

Overexpression of cNPM did not lead to marked change in the expression of ATG12

Colell et al (2007) reported that up-regulation in the expression of ATG12 would cause increase in autophagic activities. However, our result showed that there was no marked change in the expression level of ATG-12 between GFP, GFP-wtNPM and GFP-cNPM overexpressing cells.
DISCUSSION

A high LC3-II to LC3-I ratio indicates high autophagic activities and vice versa. Our data showed that GFP-overexpressing cells always demonstrated higher ratio than GFP- and GFP-overexpressing cells. These observations may imply that overexpression of cNPM promote autophagic activities especially when there is presence of cytotoxic stress.

Our data obtained here demonstrated that increased autophagic activities corresponded to high expression level of GAPDH in GFP-cNPM overexpressing cells. Moreover, it is proven that cNPM plays roles in inhibiting caspase-6 and -8 (Leong, 2005) as well as enhanced the release of cytochrome c (Tan B.X., private communication). Thus, these results may imply that after the releasing of cytochrome c; cNPM, which retards caspase activation, might play a role in up-regulating the expression of GAPDH subsequently leading to the elevation in autophagy, which is accordance to the model proposed by Colell and her colleagues (2007).

Our result obtained has shown that there is no marked difference in the expression of ATG-12, although overexpression of cNPM elevated the expression of GAPDH. This observation may suggest that cNPM might influence the expression of other ATG family members such as Beclin-1, ATG5, ATG7, ATG10 etc. (Levine and Yuan, 2005; Kundu and Thompson, 2005; Ferraro and Cecconi, 2007) instead of ATG12.

There is no error bar in this investigation due to time constraint. Thus, repeated experiments need to be performed in order to further confirm the results. Besides that, treatment of GAPDH inhibitors, or knocking down of gene encoding GAPDH from GFP-cNPM overexpressing cells can be performed in order to further investigate that GAPDH plays a role in enhancing autophagic activities. Besides that, expression of other ATG family members can be studied to identify ATG molecules which are responsible for increasing autophagic activities.

In this study, we were not able to determine that the enhanced autophagic activities will lead to cell death. Thus, more experiments need to be done in order to find out the relationship of autophagy and cell death.

REFERENCES

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